Unintended Effects of Anti-Hyperglycaemic Drugs Studied in Population-Based Cohorts

Annemariek Driessen

THE MAASTRICHT STUDY

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PROEFSCHRIFT

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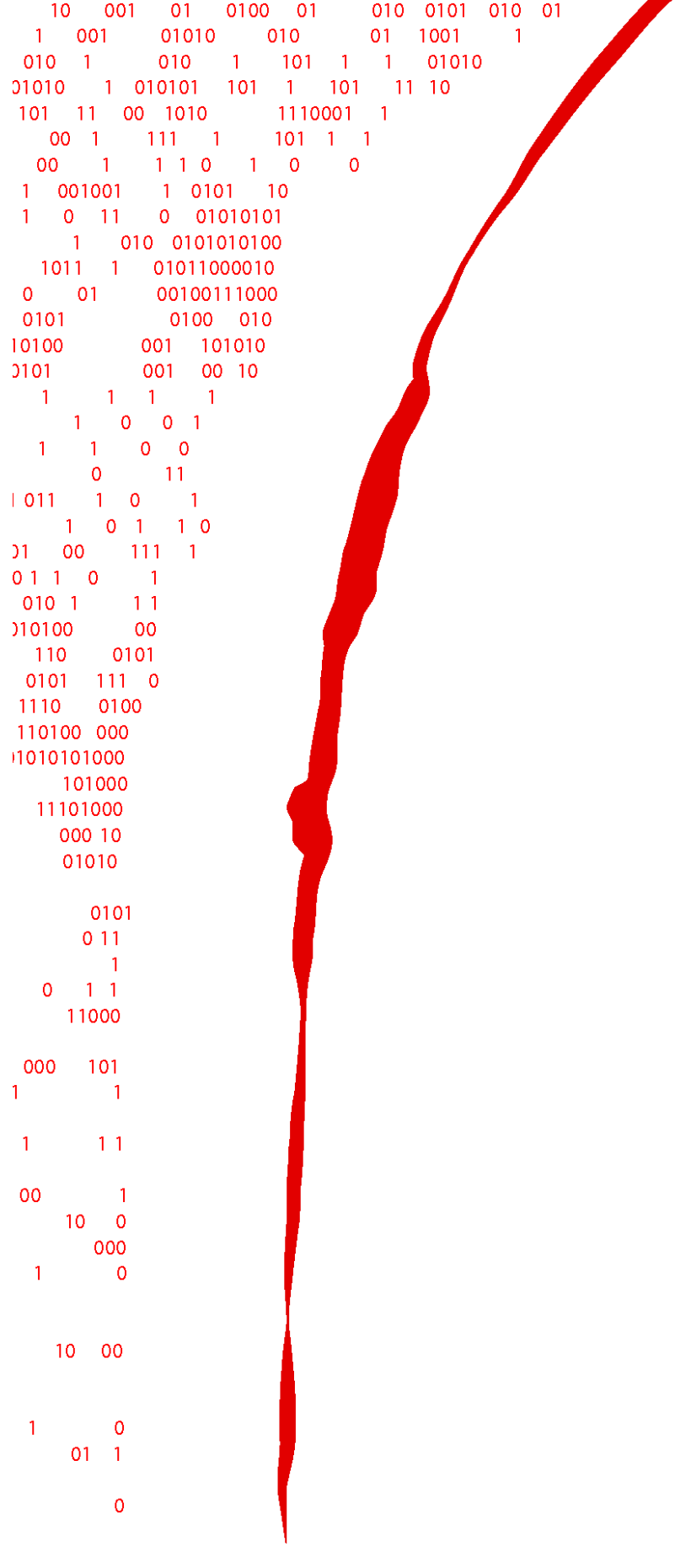
Contents

List of Abbreviations

AGE	Advanced glycation end product
ATC	Anatomical Therapeutic Chemical
BMD	Bone mineral density
BMI	Body mass index
CEL	N(ε)-(carboxyethyl)lysine
cfPWV	Carotid-femoral pulse wave velocity
CI	Confidence interval
CML	N(ε)-(carboxymethyl)lysine
COPD	Chronic obstructive pulmonary disease
CPRD	Clinical Practice Research Datalink
CVD	Cardiovascular disease
DDD	Defined daily dose
DPP4-I	Dipeptidyl peptidase 4 inhibitor
DXA	Dual-energy X-ray absorptiometry
EGFR	Estimated glomerular filtration rate
EHD	Electronic healthcare database
FRAX	Fracture risk assessment tool
GI	Gastrointestinal
GIP	Gastric inhibitory polypeptide
GIP	Glucose dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide 1
GLP1-RA	Glucagon-like peptide 1 receptor agonist
GP	General practitioner
GPRD	General practice research database
HbA1c	Glycosylated hemoglobin type A1c
HR	Hazard ratio
HR-pQCT	High-resolution peripheral quantitative computed tomography
ICD	International classification of diseases and related health problems
IOF	International osteoporosis foundation
IQR	Interquartile range
IR	Incidence rate
IRR	Incidence rate ratio
MAP	Mean arterial pressure
MAR	Missing at random
MCAR	Missing completely at random
MI	Multiple imputation
MPR	Medication possession rate
NHDR	National Hospital Discharge Register
NHSR	National Health Services Register

NIAD	Non-insulin anti-diabetic drug
NSAID	Non-steroidal anti-inflammatory drugs
OGTT	Oral glucose tolerance test
OR	Odds ratio
PS	Propensity score
PPAR-γ	Peroxisome proliferator-activated receptor-γ
RAAS	Renin angiotensin aldosterone system
RCT	Randomised controlled trial
RR	Relative risk
SAF	Skin autofluorescence
SD	Standard deviation
SU	Sulfonylurea derivative
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TBS	Trabecular bone score
TZD	Thiazolidinedione
UK	United Kingdom
vBMD	Volumetric bone mineral density



Chapter 1

General Introduction

Pharmacoepidemiology

Pharmacoepidemiology studies the use and effects of medications in large numbers of people. It frequently utilizes large "real-world" electronic healthcare databases (EHDs) that often include millions of patients and multiple years of observations. These EHDs contain longitudinal data on patient characteristics as well as medical diagnoses and sometimes also life-style variables, like body mass index (BMI), smoking status and alcohol use. More recently, longitudinal drug prescription history has also been added to these databases. With these databases population-based cohorts can be created to study exposure - outcome associations.

Examples of EHDs include the Clinical Practice Research Datalink (CPRD) (www.cprd.com), which is representative for the United Kingdom (UK) population, and the Danish National Health Services Register (NHSR)[1]. The NHSR contains data on 98% of all Danish residents and can be linked to the national hospital register[2] as well as to the national prescription registry[3]. The PHARMO Record Linkage System database (www.pharmo.nl) is a Dutch EHD, which holds drug dispensing data of more than four million Dutch residents, representing 25% of the Dutch population. This database is linked to different registers, including the national hospital discharge register.

EHDs are particularly useful when studying rare adverse events which cannot be detected in randomised controlled trials (RCTs), due to the restricted number of included patients. Consequently, with use of EHDs adverse effects with a low incidence rate can be detected. Moreover, the longer follow-up time in EHDs makes it possible to study long-term adverse effects. Another advantage of EHDs over RCTs is that when studying real-life data, adherence to treatment rates may better represent actual medication intake, while RCTs tend to overestimate adherence[4]. Additionally, RCTs often apply strong in- and exclusion criteria which consequently result in a selected population. This therefore not always reflects the real-life situation where patients have comorbidities and use other drugs concomitantly. With the use of EHDs this can be taken into account. One of the drawbacks of using observational data is the possibility for bias and confounding, which might seriously distort the effect estimates. RCTs suffer less from this problem as patients are randomised to one of the investigated treatment arms.

When interpreting the results from a pharmacoepidemiological study, one has to take the potential sources of bias and confounding into account as well as the proposed biological or pharmacological mechanism which underlies the studied effect. A study investigating statin use and risk of hip fracture found a 45% reduced risk of fracture already after 30 days of statin use[5]. The hypothesized mechanism included an increase in bone formation and inhibition of osteoclast activity. Bisphosphonates, drugs used to increase bone mineral density (BMD), inhibit the osteoclast activity as well. However, for bisphosphonates it is known to take

≥18 months (for non-vertebral fractures) to reduce fracture risk[6]. Re-analysis of the data showed that the choices made in the study design had resulted in this artificial reduced risk of fractures with statin use[7]. To overcome this problem one first has to hypothesize how the slope of the hazard function will look like where after this can be studied in multiple ways taking the time of a possible biological effect into account. Methods to study this include analyses where the time since the most recent prescription is studied. Additionally, average, cumulative dose and continuous duration of use should be studied as well as ever versus never exposure.

Osteoporosis and diabetes

Osteoporosis is defined as a systematic bone disease characterized by low bone mass and deterioration of the microarchitecture of the bone, leading to bone fragility and propensity to fracture[8]. It has been estimated that about 22 million women and 3.5 million men suffered from osteoporosis in Europe in 2010[9]. Estimates for the Netherlands showed a prevalence of 16.1 per 1000 women and 1.9 per 1000 men in 2007[10]. Osteoporosis can occur without a known underlying cause, and is diagnosed in clinical practice by the presence of a fragility fracture or using BMD criteria. BMD is the amount of bone mass per unit area (areal density, g/cm^2), and can be measured in vivo by densitometry techniques[11]. Dual-energy X-ray absorptiometry (DXA) provides a two-dimensional areal value and is influenced by bone size as well as the true density[11,12].

The BMD criteria for diagnosis were developed by the WHO on the basis of epidemiological data that describe the normal distribution of BMD in a reference population comprising healthy young adults[13]. According to these criteria, a spine, total hip or femoral neck BMD of 2.5 standard deviations (SDs) or more below the reference mean (T-score ≤-2.5) defines osteoporosis. Osteopenia—low bone density or mass—is diagnosed by a T-score between -1.0 and -2.5 and a normal BMD by a T-score ≥-1.

Diabetes is a chronic disease characterized by high blood glucose levels[14]. Diabetes is diagnosed based on a fasting glucose ≥7.0 mmol/l or a 2-h plasma glucose ≥11.1 mmol/l[15]. The estimated total number of patients with diabetes world-wide was 171 million in 2000[16]. Projections indicate that the number of patients will increase to 366 million in 2030. The estimated prevalence of diabetes in the Netherlands was 61.8 per 1000 women and 66.1 per 1000 men in 2014[17]. About 90% of the patients with diabetes have type 2 diabetes mellitus (T2DM) which is characterized by insulin resistance and/or abnormal insulin secretion. Patients with T2DM need oral anti-hyperglycaemic drugs and/or insulin to control their blood glucose levels[18].

T2DM and fracture risk

T2DM has been associated with an increased risk of fracture despite the fact that patients with T2DM often have a normal or even increased BMD[19]. It is hypothesized that this increased fracture risk is the consequence of an increased falling risk and reduced bone quality and bone strength[20,21].

Older patients with T2DM have shown to fall more often than age members without T2DM[22]. In contrast, younger patients with T2DM did not show a difference in the fall frequency as compared to patients without T2DM[23]. Nevertheless, younger patients with T2DM showed a worse performance of a functional mobility test as compared to people of the same age without T2DM, which suggests that the fall risk in young patients with T2DM might be increased as well. Complications of T2DM, like neuropathy[24] and retinopathy[25] have been associated with and may attribute to an increased risk of falling. In addition, patients with T2DM use on average nine different medications concomitantly[22]. Concomitant use of four medications has been associated with an increased risk of falling[26].

DXA measurements have extensively been used to measure BMD in persons with and without T2DM. High resolution peripheral quantitative computed tomography (HR-pQCT) is a relatively new technique which can be used to measure 3D bone density of the distal radius and tibia[27]. In addition, HR-pQCT allows the in vivo assessment of bone microarchitecture as well as calculation of biomechanical properties of the distal radius and tibia using microfinite element analysis[27]. Up to date, the HR-pQCT analyses in patients with T2DM are not uniform. An increased cortical porosity has been reported when comparing persons with and without T2DM[28,29]. However, other studies reported no differences when comparing HR-pQCT parameters between persons with and without T2DM[30-32]. In other studies, an increased cortical porosity was found only for patients with T2DM and microvascular complications[33] or in patients with T2DM and a fragility fracture compared to patients with T2DM without a fragility fracture[34].

Anti-hyperglycaemic drugs might also affect bone metabolism. For instance, use of thiazolidinediones (TZDs) has been associated with an 1.3 times increased risk of osteoporotic fracture as compared to patients using other anti-hyperglycaemic drugs[35]. TZDs are peroxisome proliferator-activated receptor (PPAR)-γ agonists and they improve glycemic control via the activation of PPAR-γ. However, they also activate PPAR-γ on mesenchymal stem cells and consequently the differentiation of osteoblasts is shifted towards adipocytes[36,37]. This might then result in a lower BMD and hence increased fracture risk.

The use of insulin has also been associated with fracture risk. In vitro studies have suggested an anabolic effect of insulin on bone. However, an increased fracture risk was seen in observational studies[38]. One of the suggested explanations is that use of insulin is associated with an increased risk of hypoglycaemia and thereby an

increased risk of falling, which then might increase fracture risk[20]. Another explanation is the fact that patients who use insulin for T2DM often have a longer disease duration and therefore also a longer hyperglycaemic exposure. Hyperglycaemic exposure has been associated with advanced glycation end products (AGEs) which can bind to collagen, and thereby might increase bone fragility and potentially increase fracture risk[39].

Incretin agents, including dipeptidyl peptidase 4 inhibitors (DPP4-Is) and glucagon-like peptide 1 receptor agonists (GLP1-RAs), are a relatively new class of anti-hyperglycaemic drugs. Both GLP1-RAs and DPP4-Is prolong the short half-life of the incretin hormone glucagon-like peptide 1 (GLP-1). GLP1-RAs do this directly as they have an increased resistance to the degradation by the enzyme dipeptidyl peptidase 4 (DPP4), whereas DPP4-Is increase GLP-1 indirectly via the inhibition of DPP4[38].

In vitro studies have suggested that these agents might have a beneficial effect on fracture risk. GLP-1 receptors have been found on osteocytes and osteoblasts, and different mechanisms have been proposed how an increased level of GLP-1 might result in a decreased fracture risk[40-42]. A first meta-analysis of adverse effects data of clinical trials indeed showed a 40% reduced risk of fractures[43]. However, observational data from population-based cohorts is lacking.

The Maastricht Study

The Maastricht Study is an observational prospective population-based cohort study which is currently collecting data from participants who live in the southern part of the Netherlands[44]. The Maastricht Study focuses on the aetiology, pathophysiology, complications and comorbidities of T2DM and is characterized by an extensive phenotyping approach. This includes extensive measurements on cardiovascular risk factors, diabetic complications, other chronic diseases, lifestyle, (psycho-) social factors as well as extensive biobanking. Additional to the current cross-sectional data, longitudinal drug dispensing data are available within the Maastricht Study, which makes it possible to perform pharmacoepidemiological studies.

Missing data and confounding

Missing data is an issue in almost every research, including observational research using EHDs or Maastricht Study data. Standard statistical techniques, such as regression, cannot handle missing data and as a consequence all observations with

at least one missing variable are deleted during the analysis (a so called "complete-case analysis"). This is inefficient and might also result in biased estimates when the data are not missing completely at random (MCAR)[45].

When data are missing due to an ill employee or a broken machine data are classified as MCAR[46]. The missing data are completely independent from other measured or unmeasured data[46]. When the missingness is conditional on other measured data, data are classified as missing at random (MAR). The probability of missingness does then not depend on the true values for the missing observations but only on other observed variables[46]. For example, patients with a high BMI might be less able to perform a physical test. Their data might therefore be more likely to be missing. Additionally, it might be assumed that patients with a higher BMI score less on a performance test. Therefore the missing data, from the patients with a high BMI, might be lower than the observed values. If BMI can then be used to fully explain the missingness of the physical test data, the data are said to be MAR. When missing data are neither MCAR nor MAR, missing data are classified as missing not at random[46].

Multiple imputation (MI) is a statistical technique which can be used to deal with missing data by replacing the missing data with imputed values, creating multiple complete datasets. The observed data are used to impute the missing data taking into account the correlations between all variables used in the imputation model[45]. The different complete datasets are then analysed and the results combined to get valid inferences. If data are MCAR, a complete-case analysis will not result in biased estimates, it will only be inefficient. However, MCAR is not very likely in practice. When data are MAR, a complete-case analysis might result in biased estimates, whereas the estimates based on MI are unbiased and more efficient[45].

Another important aspect to occur in observational research is confounding. When analyses are not adjusted for confounding factors, it might result in distorted treatment effects. There are different techniques to deal with confounding. Propensity scores (PSs) are one of them. PSs were introduced by Rosenbaum and Rubin[47] in the eighties. However, they have not been widely used until 2000[48]. A PS is the probability of being exposed given the values of the measured confounding variables[47]. The use of a PS creates a balance based on the measured confounders between the groups under study[49]. Not much research has evaluated the effects of using PSs in combination with MI on estimated treatment effects in time-fixed analyses.

Objectives of this thesis

The overall objective of this thesis was to study the unintended effects of anti-hyperglycaemic drugs in population-based cohorts. This thesis consists of three main parts: 1) evaluation of the association between use of incretin agents, both DPP4-Is and GLP1-RAs, and risk of fracture; 2) an assessment of the usefulness of Maastricht Study data to evaluate unintended effects of diabetes medication, in particular of bone strength parameters; and 3) to investigate the effect on treatment estimates when combining different techniques to handle missing data with propensity score analyses.

Outline

Chapter 2 comprises seven studies assessing fracture incidence rates and the risk of fractures when using incretin agents. It starts with a study on the incidence of fractures in Denmark in 2011 using data from the Danish NHSR. In this study we aimed to estimate fracture site specific incidence rates and to compare those to earlier reported imputed incidence rates. In Chapter 2.2 and 2.3 the association of DPP4-Is and fracture risk was studied using CPRD and Danish NHSR data, respectively. The association of GLP1-RAs and risk of fracture was evaluated using CPRD and the Danish NHSR data in Chapter 2.4 and 2.5. In addition the results of Chapter 2.2-2.5 have been meta-analysed, to further study the association between use of DPP4-Is and GLP1-RAs. This is presented in Chapter 2.6. To overcome the limitation of a restricted duration of follow-up of the data, Chapter 2.7 describes a sensitivity analysis of updated data from Chapter 2.2. In this chapter we extended the follow-up period from 2007-2012 to 2007-2015 in order to better evaluate the effects of prolonged DPP4-I use on fracture risk.

Chapter 3 consists of three studies which are performed using data from the Maastricht Study. It starts with a study investigating the representativeness of the Maastricht Study with respect to prescription data when compared to the national population. In Chapter 3.2 and 3.3, we studied the relationship of anti-hyperglycaemic drugs, as measured by longitudinal prescription data, and specific outcomes measured in the Maastricht Study. In Chapter 3.2 we examined whether use of insulin as compared to non-insulin use was associated with different bone architectural and strength parameters, as measured by HR-pQCT. In Chapter 3.3 we studied whether metformin has beneficial effects on arterial stiffness measured by carotid-femoral pulse wave velocity and what the role of AGEs was in this potential association.

Chapter 4 consists of a study that evaluated what the effect on treatment estimates is when different techniques to deal with missing data are combined with propensity score analysis in time-fixed time-to-event data. This thesis concludes with a general discussion of the results, prospects for future research and some methodological considerations.

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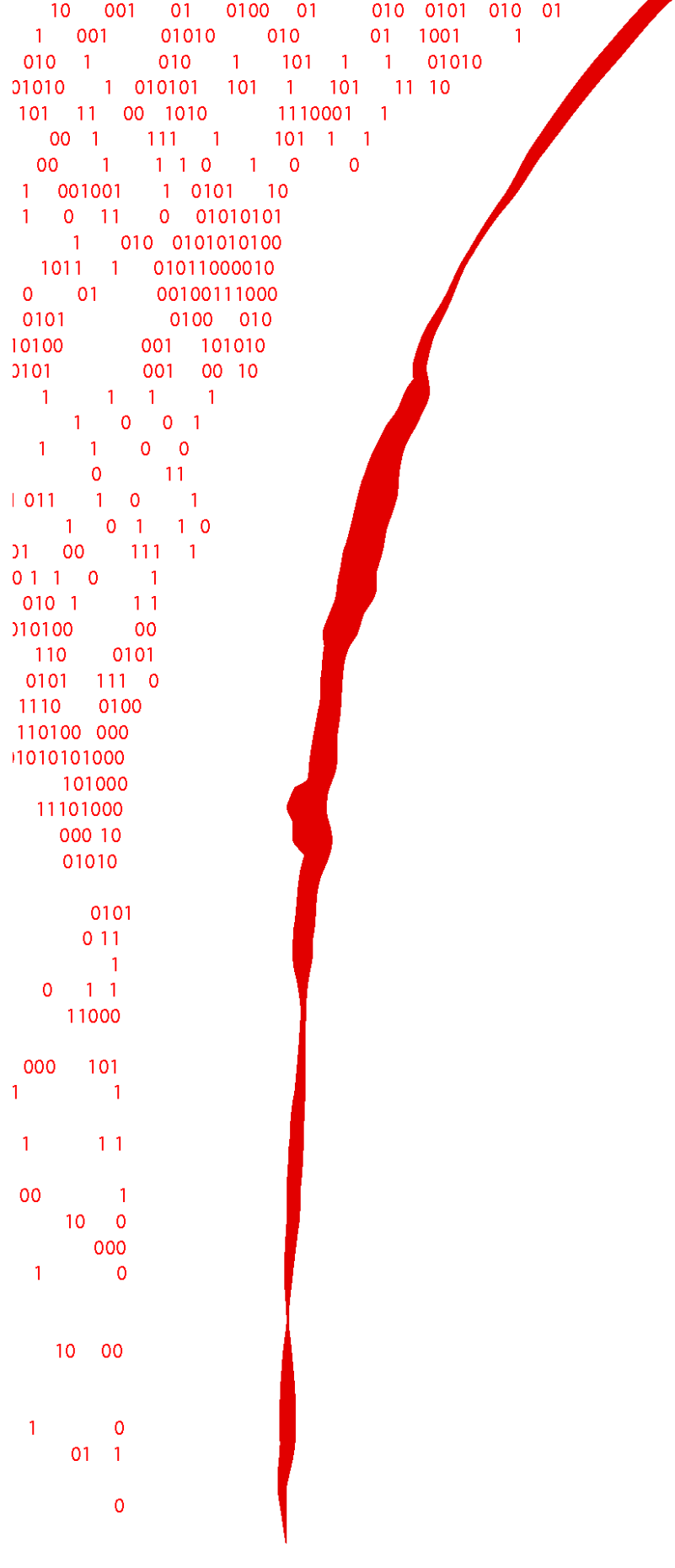

Chapter 2

Fracture risk and use of anti-hyperglycaemic drugs

Chapter 2.1

The epidemiology of fractures in Denmark in 2011

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JPW van den Bergh, B Abrahamsen, P Vestergaard, F de Vries

Osteoporos Int. 2016;27(6):2017-25

Abstract

Introduction
Osteoporosis is a major public health burden through associated (osteoporotic) fractures. In Denmark, incidence rates (IRs) of hip fracture are widely available. However, there is limited data about other fracture sites. A recent report could only provide imputed IRs, although nationwide data is readily available in electronic healthcare databases. Therefore our aim was to estimate fracture site specific IRs for Denmark in 2011 and to compare those to the previously reported imputed data.

Methods
Data from the Danish National Hospital Discharge Register was used to estimate age- and gender-specific IRs for any fracture as well as for different fracture sites in the Danish population aged 20 years and older in 2011. Hip fracture IRs were stratified to sub-sites and IRs were determined for all hip fractures which were confirmed by surgery.

Results
The total number of incident fractures in 2011 was 80,760 (IR: 191, 95% confidence interval (CI): 190-192 (per 10,000 person-years), of which 35,398 (43.8%, IR: 171, 95% CI: 169-173) occurred in men and 45,362 (56.2%, IR: 211, 95% CI: 209-213)) in women. The majority of the fractures occurred in the population aged 50 years and older (n=50,470, IR: 249, 95% CI: 247-251). The numbers of any and hip fracture were lower than the previously imputed estimates, whereas the number of forearm fractures was higher.

Conclusion
We showed age- and gender- specific fracture rates for any fracture as well as for different fracture sites. The IRs of most fracture sites increased with age. Estimating the number of fractures for Denmark based on imputation of data from other countries led to both over- and underestimation. Future research should therefore focus on how to improve those imputations as not all countries have nationwide registry data.

Introduction

Osteoporosis is a major public health burden through associated (osteoporotic) fractures. In 2010, an estimated number of 66,000 incident fractures (12,000 hip fractures) occurred in the Danish population aged 50 years and older[1]. The associated costs of osteoporosis were estimated at €1,055 million for Denmark, 2010[1]. Estimated costs of osteoporotic fractures have been projected to increase by about 50% in 2025 due to ageing of the population[2].

An increasing number of studies have shown that secular trends in the incidence of hip fractures have levelled off or started to decline[3,4]. A call to update the data for as many countries as possible has been made[3]. Hip fracture incidence rates (IRs) have been estimated in different periods for the Danish population[5,6] and IRs of distal forearm fracture are available for the period 1976-1984[7], and for 2010[8].

The International Osteoporosis Foundation (IOF) estimated the country-specific burden of osteoporosis and the number of incident fractures in 2010 for individuals aged 50+ in Denmark[1]. Danish hip fracture IRs for 2004 were available from health registries which contain data on both in- and outpatient treatments[9]. IRs for other fracture sites were not available and therefore Swedish data from 1987-1994 were used[10]. All radiography referrals that come to medical attention are recorded for Malmö, Sweden. Age- and sex- specific incidence rate ratios (IRRs) were calculated for the different fracture sites as compared to hip fracture in this population[10]. The estimated IRRs were then applied to the Danish hip fracture IRs, assuming that the age- and sex- specific IRRs were equal in Sweden and Denmark[1]. The IRs of forearm fractures in 2010 have recently been estimated for Denmark[8] using health registry data, which has shown a high validity[11], and these IRs were somewhat higher than the imputed IRs by the IOF[1].

Danish IRs are available for hip[5,6] and forearm fractures[7,8]. However, there are no data on IRs of other fracture types. It has been shown for forearm fractures that imputation based on data from other countries might underestimate the real IR[8], but this has not been examined for other fracture types. Therefore the objective of our study was to estimate fracture site specific IRs for Denmark in 2011 and to compare those to the previously reported imputed data.

Methods

Source population

In Denmark, the extensive nature of registers, covering contacts to the health sector, offers good possibilities for studies on the occurrence of fractures[11]. Using the unique 10-digit civil registry number that is assigned to all Danish citizens shortly after birth, a

complete hospital discharge history can be established for each individual, and valid linkage between population-based registries can be obtained[12]. Data on all changes in vital status, including change of address and date of death for the entire Danish population has been registered since 1968 in the Civil Registration System. The Danish National Hospital Discharge Register (NHDR)[13] was founded in 1977 and covers all inpatient contacts from 1977 to 1994, and from 1995 furthermore includes all outpatient visits to hospitals, outpatient clinics, and emergency rooms. The reliability of Danish national fracture records has shown to be high, with a concordance of 94% for hip, 84% for forearm and 83% for humerus fractures between self-reported and registered fractures in female health professionals[11]. This was not a clinical trial and ethics committee approval was not required.

Study design

Patients were included when they were diagnosed with a fracture, high- or low trauma, in 2011 and aged 20 years or older. Any fracture was determined by the following International Classification of Diseases and Related Health Problems (ICD) 10 codes: S02, S12, S22, S32, S42, S52, S62, S72, S82, S92, T02, T08, T10 and T12. We investigated the following fracture sites: skull (S02), clinical symptomatic vertebral (S12, S22.0, S22.1, S32.0, T08), ribs (S22.2-S22.9), pelvis (S32), clavicle (S42.0), scapula (S42.1), humerus (S42.2-S42.4), forearm (S52), carpal (S62.0, S62.1), hip (S72.0-S72.2), femur unspecified (S72.3-S72.9), patella (S82.0), tibia/fibula (S82.1-S82.4), ankle (S82.5, S82.6, S82.8), and foot (S92). Unspecified fracture consisted of all other fracture ICD10 codes. Major osteoporotic fracture was determined as a hip, humerus, forearm or clinical symptomatic vertebral fracture according to the WHO definition[14]. Hip fracture was further stratified by the location of the fracture: neck, pertrochanteric or subtrochanteric (ICD-10: S72.0, S72.1 and S72.2, respectively). Surgery codes “KNFB” and “KNFJ4-9”[15] were used to determine which hip fractures were confirmed by surgery within 10 days after the date of fracture.

The population demographics of the background population in 2011 were obtained online from Statistics Denmark (www.statistikbanken.dst.dk). IRs (number of fractures / 10,000 person-years) were calculated by dividing all cases of a first recorded fracture during 2011 over the total number of persons alive at July 1, 2011 and aged 20 or older. To overcome the potential problem of counting the same fracture twice, all fractures with a record of a previous fracture (of the same type or coded as unspecified) in the six months before the fracture date in 2011 were excluded. Age- and gender- specific IRs were estimated as well as IRs for the above defined fracture sites. Women-to-men IRRs were determined by dividing the women IR over the men IR. For the different fracture types the first fracture in that specific category was used to calculate the IR.

Results

The total number of incident fractures in people aged 20 years and older in 2011 was 80,760 (IR: 191, 95% confidence interval (CI): 190-193 per 10,000 person-years (py)), of which 45,362 (56.2 %, IR: 211, 95% CI: 209-213) occurred in women and 35,398 (43.8 %, IR: 171, 95% CI: 169-173) in men, Table 2.1.1. The majority of the fractures occurred in the population aged 50 years and older (n=50,470, IR: 249, 95% CI: 247-251), Table 2.1.1. When patients with a history of a fracture in the six months before were not excluded, the total number of incident fractures in people aged 50 years and older was 4.5% higher (n=52,745). Until the age of 50, fractures occurred more often in men than in women (women to men IRR: 0.60, 95% CI: 0.59-0.62). Whereas after the age of 50, the majority of the fractures occurred in women (IRR: 1.89, 95% CI: 1.85-1.92). Figure 2.1.1 shows that the IRs strongly increased in both men and women after age 75.

The total number of incident major osteoporotic fractures was 35,102 of which 82% occurred in the population aged 50 years and older. Women accounted for two-third of the major osteoporotic fractures and their IR was 2.5 times higher than the IR for men aged 50+, Table 2.1.2. Men and women showed a comparable IR until the age of 50, thereafter the IRs started to diverge until the age of 75, Figure 2.1.2.

The following sites showed homogeneous patterns of fracture IRs with age: femur, hip, humerus, pelvis, clinical symptomatic vertebral, rib, major osteoporotic fracture and any. There was an exponential increase starting at the 50-54 age category, Figure 2.1.2. The IR of forearm fractures yielded a strong increase from the age of 45 years. IRs of tibia and clavicle fracture started to rise at age 75. IRs of carpal, skull and foot fracture (men only) declined with age, whereas IRs of foot fractures rose in women aged 45-59 years and decreased thereafter. The IR of ankle fractures in men was steady, in contrast to the IR for women, which rose until age 60 and then stabilized. Rib and patella fracture showed a continuous increase for both men and women. Table 2.1.2 shows the IRs and total number of fractures in the population aged 50 and older. The women-to-men IRR was highest for forearm fracture (IRR: 3.8).

Stratification to hip fracture sites did not result in different patterns, Table 2.1.3. The hip fracture IRs strongly increased from age 75. The IR was higher in women than in men and the majority (53.5%) of the hip fractures occurred in the neck of the hip. Table 2.1.4 shows the IRs of hip fractures which were confirmed by a surgery in the 10 days after the date of fracture, those IRs were about 15 to 20% lower than the total hip fracture IRs. The same IR pattern was visible as with hip fracture and hip fracture stratified by fracture location.

Table 2.1.1 Number of incident fractures in Denmark in 2011 by age and sex.

	Total			Men			Women			
Age	Number of fractures	Number of py at risk	Incidence rate per 10,000 py (95% CI)	Number of fractures	Number of py at risk	Incidence rate per 10,000 py (95% CI)	Number of fractures	Number of py at risk	Incidence rate per 10,000 py (95% CI)	IRR:W:M (95% CI)
20-24	6,436	340,005	189 (185 - 194)	4,379	173,397	253 (245 - 260)	2,057	166,608	123 (118-129)	0.49 (0.46 - 0.52)
25-29	4,507	313,457	144 (140 - 148)	2,960	157,771	188 (181 - 194)	1,547	155,686	99 (94 - 104)	0.53 (0.50 - 0.56)
30-34	4,120	336,815	122 (119 - 126)	2,641	168,862	156 (150 - 162)	1,479	167,953	88 (84 - 93)	0.56 (0.53 - 0.60)
35-39	4,539	387,575	117 (114 - 120)	2,747	194,050	142 (136 - 147)	1,792	193,525	93 (88 - 97)	0.65 (0.62 - 0.69)
40-44	5,015	397,530	126 (123 - 130)	3,040	201,319	151 (146 - 156)	1,975	196,211	101 (96 - 105)	0.67 (0.63 - 0.71)
45-49	5,673	416,527	136 (133 - 140)	3,249	210,661	154 (149 - 159)	2,424	205,866	118 (113 - 122)	0.76 (0.72 - 0.80)
50-54	5,728	367,878	156 (152 - 160)	2,739	185,107	148 (142 - 153)	2,989	182,771	164 (158 - 169)	1.11 (1.05 - 1.16)
55-59	6,501	350,093	186 (181 - 190)	2,537	174,892	145 (139 - 151)	3,964	175,201	226 (219 - 233)	1.56 (1.48 - 1.64)
60-64	6,894	356,791	193 (189 - 198)	2,441	176,655	138 (133 - 144)	4,453	180,136	247 (240 - 254)	1.79 (1.70 - 1.88)
65-69	7,265	331,398	219 (214 - 224)	2,403	162,919	147 (142 - 153)	4,862	168,479	289 (281 - 297)	1.96 (1.86 - 2.05)
70-74	5,492	226,853	242 (236 - 248)	1,720	107,393	160 (153 - 168)	3,772	119,460	316 (306 - 326)	1.97 (1.86 - 2.09)
75-79	5,109	165,202	309 (301 - 318)	1,471	74,035	199 (189 - 209)	3,638	91,167	399 (386 - 412)	2.01 (1.89 - 2.13)
80-84	5,101	117,219	435 (423 - 447)	1,341	47,938	280 (265 - 294)	3,760	69,281	543 (526 - 560)	1.94 (1.82 - 2.06)
85-89	4,665	73,215	637 (619 - 655)	1,052	24,911	422 (397 - 447)	3,613	48,304	748 (725 - 771)	1.77 (1.65 - 1.90)
90+	3,715	39,087	950 (921 - 980)	678	9972	680 (630 - 729)	3,037	29,115	1,043 (1008 - 1078)	1.53 (1.41 - 1.67)
Total	80,760	4,219,645	191 (190 - 193)	35,398	2,069,882	171 (169 - 173)	45,362	2,149,763	211 (209 - 213)	1.23 (1.22 - 1.25)
50+	50,470	2,027,736	249 (247 - 251)	16,382	963,822	170 (167 - 173)	34,088	1,063,914	320 (317 - 324)	1.89 (1.85 - 1.92)

CI: confidence interval; IRR:W:M: incidence rate ratio women to men; py: person-years.

Table 2.1.2 Incidence rate and total number of fractures stratified by gender and fracture type in people aged 50 years and older.

	Incidence rate / 10,000 py (95% CI)			Number of fractures		
Fracture type	Total	Men	Women	Total	Men	Women
Ankle	20.9 (20.3 - 21.5)	14.0 (13.2 - 14.7)	27.1 (26.1 - 28.1)	4,234	1,348	2,886
Carpal	6.4 (6.0 - 6.7)	4.8 (4.3 - 5.2)	7.8 (7.3 - 8.4)	1,295	460	834
Clavicle	8.9 (8.5 - 9.3)	10.4 (9.7 - 11.0)	7.7 (7.1 - 8.2)	1,812	998	814
Femur	6.1 (5.8 - 6.4)	4.0 (3.6 - 4.4)	8.0 (7.5 - 8.5)	1,237	385	852
Foot	21.0 (20.4 - 21.6)	14.8 (14.0 - 15.5)	26.6 (25.7 - 27.6)	4,257	1,423	2,834
Forearm	65.1 (64.0 - 66.2)	26.0 (25.0 - 27.0)	100.5 (98.6 - 102.4)	13,192	2,504	10,688
Hip	43.8 (42.9 - 44.7)	29.2 (28.1 - 30.2)	57.1 (55.7 - 58.5)	8,884	2,811	6,073
Humerus	29.1 (28.3 - 29.8)	15.9 (15.2 - 16.7)	40.9 (39.7 - 42.2)	5,893	1,537	4,356
Patella	3.3 (3.0 - 3.5)	2.2 (1.9 - 2.5)	4.3 (3.9 - 4.7)	666	211	455
Pelvis	9.7 (9.2 - 10.1)	5.4 (4.9 - 5.8)	13.6 (12.9 - 14.3)	1,962	517	1,445
Rib	7.7 (7.3 - 8.1)	10.3 (9.6 - 10.9)	5.4 (4.9 - 5.8)	1,562	991	571
Scapula	1.6 (1.4 - 1.8)	2.0 (1.8 - 2.3)	1.2 (1.0 - 1.4)	320	197	123
Skull	7.7 (7.3 - 8.1)	7.5 (6.9 - 8.0)	7.9 (7.4 - 8.4)	1,561	720	841
Tibia	8.7 (8.3 - 9.1)	6.3 (5.8 - 6.9)	10.8 (10.2 - 11.4)	1,761	612	1,149
Clinical symptomatic vertebral	10.9 (10.4 - 11.3)	10.3 (9.6 - 10.9)	11.4 (10.8 - 12.1)	2,204	989	1,215
Major osteoporotic	142.0 (140.3 - 143.6)	78.4 (76.6 - 80.1)	199.6 (196.9 - 202.2)	28,786	7,552	21,234

CI: confidence interval; major osteoporotic: hip, forearm, humerus or clinical symptomatic vertebral fracture; py: person-years.

Table 2.1.3 Incidence rates / 10,000 py for hip fractures in people aged 50 years and older stratified by age, sex and hip fracture type.

Type of hip fracture (ICD-10 code)	Neck (S72.0)		Petrochanteric (S72.1)		Subtrochanteric (S72.2)	
	Hip fracture incidence rates / 10,000 py (95% CI)					
Age	Men	Women	Men	Women	Men	Women
50-54	3 (2 - 4)	3 (2 - 4)	3 (2 - 4)	3 (2 - 3)	1 (0 - 1)	1 (0 - 1)
55-59	4 (3 - 5)	6 (5 - 7)	4 (3 - 4)	3 (2 - 4)	1 (0 - 1)	1 (0 - 1)
60-64	7 (6 - 9)	9 (7 - 10)	6 (5 - 7)	9 (8 - 10)	1 (1 - 2)	1 (1 - 2)
65-69	11 (10 - 13)	18 (16 - 20)	8 (7 - 10)	13 (11 - 14)	2 (2 - 3)	3 (2 - 4)
70-74	18 (16 - 21)	30 (27 - 33)	14 (12 - 16)	27 (25 - 30)	5 (4 - 6)	5 (4 - 6)
75-79	35 (31 - 39)	56 (51 - 61)	25 (21 - 28)	56 (53 - 60)	7 (5 - 9)	9 (7 - 11)
80-84	68 (60 - 75)	107 (100 - 115)	50 (43 - 56)	97 (90 - 103)	10 (7 - 13)	16 (13 - 19)
85-89	122 (108 - 136)	196 (184 - 208)	93 (81 - 105)	140 (129 - 150)	12 (7 - 16)	29 (24 - 34)
90+	221 (192 - 249)	282 (263 - 301)	158 (134 - 183)	232 (215 - 249)	25 (15 - 35)	55 (46 - 63)

CI: confidence interval; ICD: International Classification of Diseases and Related Health Problems; py: person-years.

Table 2.1.4 Incidence rates / 10,000 py for hip fractures which were confirmed by surgery in people aged 50 years and older stratified by age, sex and hip fracture type.

Type of hip fracture (ICD-10 code	Neck (S72.0)		Petrochanteric (S72.1)		Subtrochanteric (S72.2)	
	Hip fractures incidence rates / 10,000 py confirmed by surgery (95% CI)					
Age	Men	Women	Men	Women	Men	Women
50-54	2 (2 - 3)	2 (2 - 2)	2 (1 - 3)	1 (1 - 1)	1 (0 - 1)	1 (0 - 1)
55-59	4 (3 - 5)	4 (4 - 5)	3 (2 - 3)	2 (2 - 3)	1 (0 - 1)	1 (0 - 1)
60-64	6 (5 - 7)	7 (6 - 7)	5 (4 - 6)	4 (3 - 4)	1 (1 - 2)	1 (1 - 1)
65-69	9 (7 - 10)	15 (14 - 16)	7 (6 - 8)	8 (7 - 9)	2 (1 - 2)	2 (2 - 3)
70-74	15 (13 - 17)	25 (24 - 27)	11 (9 - 13)	15 (14 - 16)	4 (3 - 5)	4 (3 - 5)
75-79	28 (25 - 31)	47 (45 - 50)	20 (17 - 22)	30 (28 - 32)	5 (4 - 7)	7 (6 - 8)
80-84	55 (51 - 60)	89 (85 - 93)	42 (38 - 45)	62 (59 - 66)	9 (7 - 11)	13 (11 - 14)
85-89	102 (96 - 108)	162 (155 - 170)	80 (75 - 86)	122 (115 - 128)	8 (6 - 10)	24 (22 - 27)
90+	173 (165 - 182)	226 (213 - 238)	128 (121 - 136)	203 (191 - 215)	18 (15 - 21)	47 (41 - 53)

CI: confidence interval; ICD: International Classification of Diseases and Related Health Problems; py: person-years.

Our estimated number of fractures for various sites was considerably different to the estimates from the IOF. The number of incident fractures in patients aged 50 years and older (including people with a history of a fracture in the 6 months before) was 52,745, 10,488, 13,746, and 2,299 in the present study for any, hip, forearm and clinical symptomatic vertebral fracture, respectively. The IOF estimate was 25% higher for any fracture (n=66,000), 14% higher for hip fracture (n=12,000) and 27% lower for forearm fracture (n=10,000)[1]. The IOF estimate for clinical symptomatic vertebral fracture was 4.3 times higher (n=10,000) as compared to our results[1].

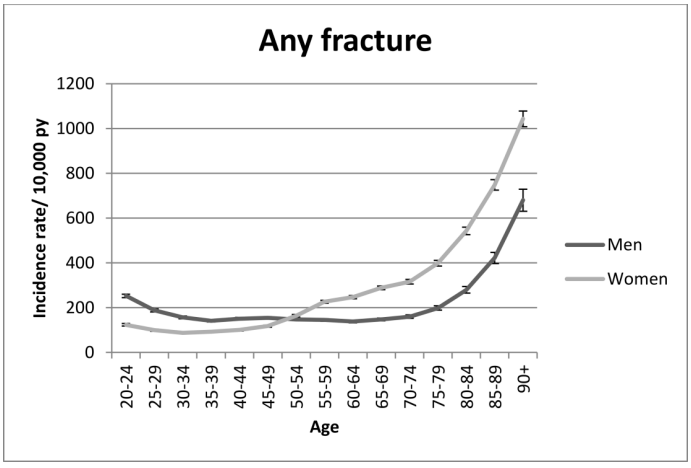
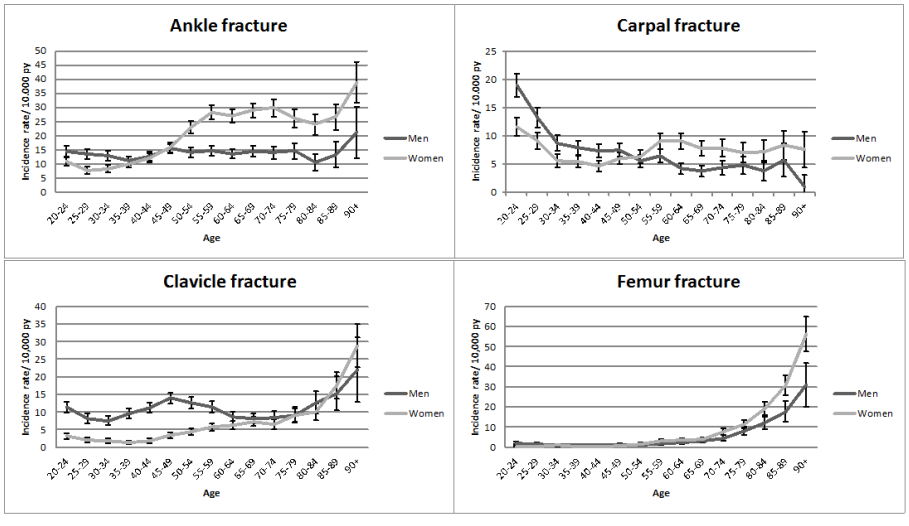
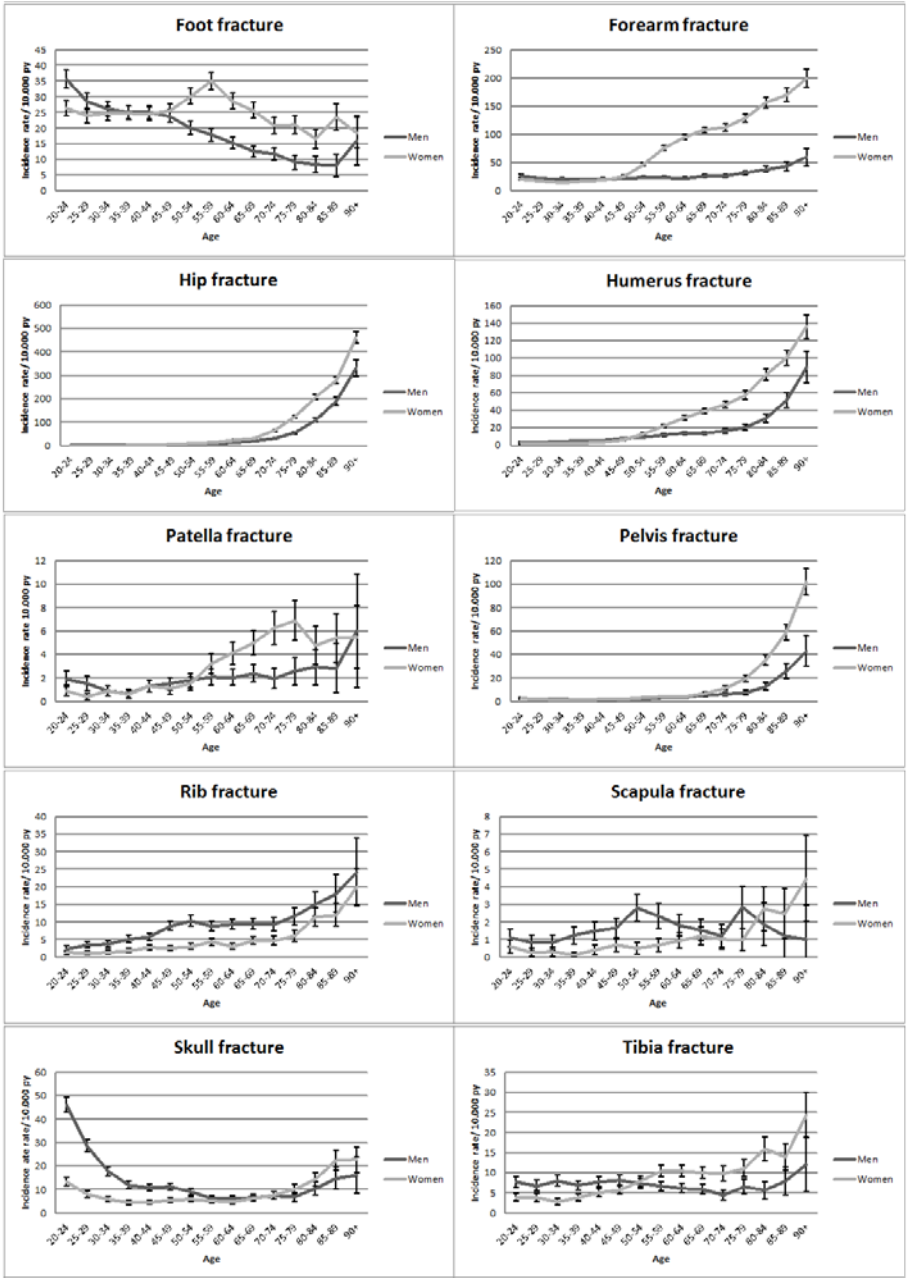


Figure 2.1.1 Incidence rates of first fracture in 2011 stratified by age and sex. py: person-years.




Foot fracture
Forearm fracture
Hip fracture
Humerus fracture
Patella fracture
Pelvis fracture
Rib fracture
Scapula fracture
Skull fracture
Tibia fracture
Incidence rate / 10.000 py
Age
Men
Women

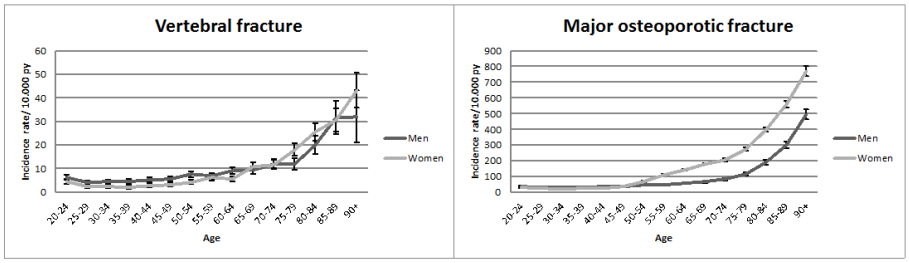


Figure 2.1.2 Incidence rates of first fracture in 2011 stratified by fracture type, age and sex. Major osteoporotic: hip, forearm, humerus or clinical symptomatic vertebral fracture.

Discussion

We have reported age- and gender- specific IRs for different fracture sites in the Danish population aged 20 years and older in 2011. The total number of incident fractures was 80,760. The IRs for any, hip, femur, humerus, pelvis, clinical symptomatic vertebral, rib, and major osteoporotic fracture increased with age, in contrast to fractures of the carpus, foot or skull, which declined with age. Until the age of 50, fractures occurred more frequently in men than in women, while this was reversed after the age of 50.

The pattern of IRs of forearm fractures of the present study are in line with the results of a study that investigated the IRs of forearm fractures in Denmark in 2010[6]. Both studies showed a steady IR up to age categories of 44 years and a rise thereafter, especially in women. However, the IRs of forearm fractures in women aged 50 to 79 year were lower (8-21 fractures / 10000 py) in the present study than in the 2010 study, which is might be due to random variation and the fact that we excluded patients with an unspecified fracture in the six months before the date of fracture in 2011[6]. The results for hip fracture are comparable with earlier results from Denmark, 2008[4]. Our results are in keeping with results for the Dutch population[16], when considering a three-fold difference in hip fracture IRs as shown earlier[17]. As compared to IRs for Norway[18], our results are similar below the age of 80, whereas our results are lower than the Norwegian IRs for age groups of ≥80. Based on a study which estimated standardized hip fracture rates for different European countries a higher hip fracture IR for Denmark was expected as compared to Norway[17]. The Norwegian study used data from 1994-2008 of a particular city to estimate IRs, while we used data from the whole country of Denmark for 2011 and this might partly explain the differences. Previous studies have demonstrated higher IRs of osteoporotic fractures in urban as compared to rural regions in Norway although this is believed principally to be with regard to forearm fractures[19,20].

The same pattern of IRs (stable until the age of 50 years, and a strong rise thereafter) was visible for any fracture, as compared to the results of a study investigating the IRs of different fracture sites in the United Kingdom, between 1988 and 1998[21]. Both studies investigated different fracture sites and those were comparable as well, except for patella and clinical symptomatic vertebral fracture, which showed a different pattern in people aged 80 and over. The IR was about 1.5-2 times higher in Denmark than in the UK, except for rib fracture which showed an equal IR. This may be explained by various reasons, such as differences in the definition of incident fracture between the studies, differences in the studied time period and differences in the used data source. Additionally, it is known from literature that IRs in Scandinavia are about 1.5-2 times higher than those in the rest of Europe[4,22,23].

The IOF recently estimated numbers of fractures in 2010 for 27 different EU countries, including Denmark[1]. This is of course helpful for an appraisal of the societal burden and the report relied on the official statistics as available. Hence, for Denmark, the IOF had only data available on hip fractures, and imputed numbers of other fracture sites using the ratios of hip to other fracture types using Swedish data of Malmo (1987-1994)[10]. The IOF estimates were 25% higher for any fracture and 14% for hip fractures as compared to the numbers estimated in the current study. In contrast, the IOF estimate for forearm fractures was 27% lower than the present number of fractures. This is in line with recent incidence rates of forearm fractures in Denmark in 2010[6], which used the same data source as in this study.

The number of clinical symptomatic vertebral fractures estimated by the IOF is about 4.3 times higher as the number of clinical symptomatic vertebral fractures in the present study. The IOF used Swedish age- and gender- specific IRRs from vertebral to hip fracture IRs based on radiography referrals. In the present study we used register data which especially for clinical vertebral fractures might be less accurate than the radiographic data. As far as we know there is no data available on how well clinical vertebral fractures are captured in the Danish registry and therefore it is difficult to compare the IOF estimate to the estimate of the present study. When comparing the present data to the data from the IOF one has to take into account that there are some differences between the studies. The IOF estimated the number of fractures for 2010, based on data from 2004 including repeat admissions[1], while we used data from 2011 and applied a washout period of six months. Hip fracture IRs have shown to decline in Denmark[4,6]. This decline in hip fracture incidence rates and the repeat admissions might explain the overestimation of the number of hip fractures. Imputation of fracture incidence rates might be improved by taking secular trends of fracture incidence rates into account.

This study has several strengths. We were able to estimate the number of fractures in 2011 for the full country of Denmark. Moreover, we were able to stratify to different fracture sites, without imputation based on data from other fracture sites or other countries. This made it also possible to compare our data to estimated IRs based on

imputed data. The reliability of Danish national fracture records has previously been addressed in the Danish Nurses Cohort Study (n=18,800), where 94% concordance was found between self-reported hip fracture in female health professionals and registered hip fracture, 84% concordance for forearm fractures, and 83% for humerus fractures[11].

Furthermore, the present results provide more accurate estimates of the number of fractures and thereby these data will assist to the planning of health services and to the estimation of fracture associated costs. And the present estimates could also be used to update the Danish algorithms for fracture risk assessment tool (FRAX). At present, the Danish version of FRAX, includes in the major osteoporotic fracture risk estimate a vertebral fracture probability that is imputed from Danish official hip fracture rates using Malmö conversion ratios, while forearm and humerus fractures are those reported by the Danish National Board of Health at the time. The present study has the advantage of using a conservative approach to reduce the risk of multiple counting of the same fracture, or including hip fracture repair and it is also used the most up to date incidence rate report.

We are aware of some limitations of this study. Our IR estimates might be somewhat conservative, because we excluded all fractures with a history of fracture of the same type in the six months before, to overcome the problem of double counting of the same fracture. Additionally, we were only able to estimate the number of vertebral fractures that came to clinical attention. This of course reflects the immediate societal burden and use of the healthcare system but patients with vertebral fractures who do not receive the diagnostic work-up including x-rays are also subject to increased morbidity and mortality[24].

In summary, we have shown age- and gender- specific fracture rates for any fracture as well as for different fracture sites. The IRs of most fracture sites increased with age. Until the age of 50 fractures occurred more in men than in women, while this was reversed in older age groups. Estimating the number of fractures for Denmark based on imputation of data from other countries led to both over- and underestimation. Future research should therefore focus on how to improve those imputations as not all countries have nationwide registry data.

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Chapter 2.2

Use of dipeptidyl peptidase 4 inhibitors for type 2 diabetes mellitus and risk of fracture

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Abstract

Introduction

Although patients with type 2 diabetes mellitus have an increased bone mineral density as compared to healthy patients, their risk of fracture is elevated. Incretins, new anti-diabetic drugs, may have a protective effect on bone mineral density. However, data on the effect of incretins on fracture risk are limited. Therefore the aim of this study was to investigate the association between the use of dipeptidyl peptidase 4 inhibitors (DPP4-Is) and the risk of fracture.

Methods

A retrospective population-based cohort study, using data from the Clinical Practice Research Datalink (CPRD) database (2007-2012), was conducted. Patients (n=216,816) with at least one prescription for a non-insulin anti-diabetic drug (NIAD), aged 18+ during data collection, were matched to one control patient. Cox proportional hazards models were used to estimate the hazard ratio of any fracture in DPP4-I users versus controls and versus other NIAD patients. Time-dependent adjustments were made for age, sex, life style, comorbidity and drug use.

Results

The actual duration of DPP4-I use was 1.3 years. There was no different risk of fracture comparing current DPP4-I users to controls (adjusted hazard ratio (adj. HR) 0.89, 95% confidence interval (CI): 0.71-1.13). There was also no increased risk comparing current DPP4-I users to other NIAD users, adj. HR 1.03 (95% CI: 0.92-1.15).

Conclusion

DPP4-I use was not associated with fracture risk fracture compared to controls and to other NIAD users. However, the duration of DPP4-I use in our database might have been too short to show an association with fracture risk.

Introduction

Osteoporosis is a common disease and a major public health burden through associated fractures. In 2010, an estimated 2.7 million hip fractures occurred worldwide, of which about 1.8 million were in women[1]. As the incidence of hip fracture continues to increase worldwide, projections indicate that the number of hip fractures occurring in the world each year will rise to 6.26 million by 2050[2]. Although patients with type 2 diabetes mellitus (T2DM) have an increased bone mineral density (BMD) as compared to controls, their risk of fracture is elevated[3]. This suggests that this elevated risk is due to reduced bone strength or quality. However, some of the anti-T2DM drugs also have been associated with an increased fracture risk, for example thiazolidinediones (TZDs)[4-6] and human insulins[7], while others, like metformin have been associated with a reduced fracture risk[8].

In vivo research has shown that glucagon-like peptide 1 receptor agonists (GLP1-RAs), a new class of anti-diabetic drugs, might have a beneficial effect on bone[9-11]. This effect might be established by increasing the level of glucagon-like peptide 1 (GLP-1) directly (via GLP1-RAs) or indirectly (via dipeptidyl peptidase 4 inhibitors (DPP4-Is) and the GLP-1 receptor which might be present on osteocytes and osteoblasts, as shown in in vitro studies[12,13]. Finally this might lead to an increase in BMD.

Data on the effects of GLP1-RAs and DPP4-Is on fracture risk are limited to a small number of patients and conflicting. A meta-analysis of randomised controlled trials (RCTs) that compared DPP4-Is with a comparator group or placebo confirmed a 40% significant reduction in the risk of fracture[14]. However, this meta-analysis had several limitations: the total number of patients were small (n=21,055), there were heterogeneous comparator groups. In contrast, a study investigating the effect of vildagliptin, a DPP4-I, on bone markers in humans did not show a change in markers representing bone resorption and calcium homeostasis[15]. The possible protective effect of DPP4-Is on risk of fracture in humans is not been well established. Therefore the aim of this study was to investigate the association between the use of DPP4-Is and the risk of fracture.

Methods

Data for this study were obtained from the Clinical Practice Research Datalink (CPRD) in the United Kingdom, previously known as the General Practice Research Database (GPRD) [www.CPRD.com]. The CPRD contains computerized medical records of 625 primary care practices in the United Kingdom, representing 8% of the population. The data recorded in the CPRD include demographic information, prescription details, clinical events, preventive care provided, specialist referrals, hospital admissions, and major outcomes since 1987. Previous studies using CPRD data have shown to be highly valid, with for example for hip fractures over 90% confirmed diagnoses[16].

We conducted a retrospective population-based cohort study. The case population consisted of all patients with at least one prescription for a non-insulin anti-diabetic drug (NIAD) and who were aged 18+ during the period of valid CPRD data collection. For this study, data collection started on June 13th 2007, the date of the first ever prescription of a DPP4-I in CPRD, and ended in August 2012. The index date was defined as the date of the first NIAD prescription since the start of the study period (i.e. the study population was a mix of incident and prevalent NIAD users).

After start of valid data collection a NIAD user was matched by sex, year of birth (within 5 years), and practice to one control. Control patients were patients who never had a prescription of a NIAD or insulin during the entire study period. The index date of the controls was set to the index date of the matched NIAD user and their period of follow-up was divided into intervals of 90 days. Each patient was followed from his or her index date to the end of data collection, the date of transfer out of the practice area, or the patient`s death, whichever came first.

The follow-up time of the NIAD users was divided into intervals based on the NIAD and insulin prescriptions, i.e. for every prescription a new interval was created. When there was a washout period of 90 days, an interval was classified as “past NIAD use”, until end of follow-up, or a new prescription of an anti-diabetic drug, whichever came first. Otherwise an interval was classified as “current NIAD use”.

All DPP4-I exposed intervals were classified, according to the time since the most recent prescription, as current (1-90 days), recent (91-180 days), or past (over 180 days) use. At every DPP4-I current use interval, the cumulative prescribed DPP4-I dosage, in sitagliptin dose equivalents, was reviewed and divided by the DPP4-I treatment time (difference in time between the start of the first and last DPP4-I prescription) to estimate the average daily DPP4-I dose. Defined daily doses were used to calculate the sitagliptin dose equivalents[17]. For all current DPP4-I users, a half-year medication possession rate (MPR) was estimated at the date of their latest DPP4-I prescription. The prescribed quantity and the written dosage instruction were used to estimate the duration of treatment. The MPR was determined as the estimated duration of treatment divided by the actual duration (i.e. 182 days). When prescriptions were overlapping, the overlapping days were added to the estimated duration of treatment. A MPR of 80% or more was used as cut-off to categorize patients as compliant (MPR ≥80%) or not compliant (MPR <80%).

Patients were followed up from the index date to either the end of data collection, the date of transfer of the patient out of the practice area, the patient's death, or the fracture type of interest, whichever came first. Fractures were classified by use of read codes. We used the following categories to classify fractures: any, hip, radius/ulna, vertebral, and major osteoporotic fracture. A major osteoporotic fracture was defined as a fracture of the hip, vertebrae, radius/ulna or humerus according to the WHO definition[18].

The presence of risk factors was assessed by reviewing the computerized medical records for any record of a risk factor prior to the start of an interval. The following potential confounders were determined at baseline: sex, body mass index (BMI), smoking status and alcohol use. All other risk factors that were considered in this study were determined time-dependently (i.e. at the start of each interval). We considered the following potential confounders: age, glycosylated hemoglobin type A1c (HbA1c), falls in 7-12 months before the start of an interval, a history of chronic obstructive pulmonary disease, previous fracture, rheumatoid arthritis, hypothyroidism, hyperthyroidism, cancer, retinopathy, neuropathy, congestive heart failure and secondary osteoporosis (hypogonadism or premature menopause (<45 year)). In addition, the following drug prescriptions in the 6 months prior to the start of an interval were considered as a potential confounder: oral glucocorticoids, cholesterol lowering drugs, antidepressants, anxiolytics or hypnotics, antipsychotics, anti-Parkinson drugs, antihypertensives (beta-blockers, thiazide diuretics, renin angiotensin aldosterone system inhibitors, calcium channel blockers, loop diuretics), antiarrhythmics, opposed hormone replacement therapy, calcium, bisphosphonates, vitamin D, raloxifene, strontium ranelate, calcitonin, parathyroid hormone, insulin and TZDs.

Regression analysis with Cox proportional hazards models (SAS 9.2, PHREG procedure) was used to estimate the fracture rate of current DPP4-I users compared to control patients (non-NIAD users). This analysis was stratified by major osteoporotic, hip, radius/ulna and clinically symptomatic vertebral fracture, as well as sex and different age categories. As a second analysis the fracture risk of current DPP4-I users was compared to other current NIAD users. The analysis was stratified by major osteoporotic, hip, radius/ulna, and vertebral clinically symptomatic fracture, as well as sex and different age categories. In different analyses we stratified current DPP4-I use by average daily dose, cumulative DPP4-I use and the estimated half-year MPR (low MPR <80% and high MPR ≥80%). In all analyses potential confounders were included if they independently changed the beta-coefficient for current DPP4-I exposure by at least 5%, or when consensus about inclusion existed within the team of researchers, supported by clinical evidence from literature.

Results

Table 2.2.1 shows the baseline characteristics of the DPP4-I users, the NIAD users, and their matched controls. In total we included 216,816 NIAD users and the same number of controls. The mean age of NIAD users and the controls was 61 years in both groups (standard deviation (SD) 21.0 for both groups). The DPP4-I users were slightly younger at baseline, i.e. 59 years (SD 16.0). The percentage of women was 47.3% within the NIAD user group and the matched controls and 43.0% in the DPP4-I user group. The

median follow-up time (from start of follow-up to end of data collection) was 3.7 years (Inter quartile range (IQR), 1.61-5.22) and 3.95 years (IQR, 1.79-5.22) for the NIAD users and controls, respectively. The DPP4-I users had a median follow-up time (from the first NIAD prescription until the end of data collection) of 5.0 years (IQR, 2.95-5.16) and a median duration of actual use (from the first DPP4-I prescription until the last DPP4-I prescription) of 1.04 years (IQR, 0.48-1.92).

Table 2.2.1 Baseline characteristics of DPP4-I users, NIAD users and their matched controls[a].

Characteristic	DPP4-I users[b] N=22,510	NIAD users[c] N=216,816	Controls[d] N=216,816
Median follow-up time, years [IQR]	5.0 [2.95 – 5.16]	3.7 [1.61 – 5.22]	3.95 [1.79 – 5.22]
Actual duration of DPP4-I use, years [IQR]	1.04 [0.48 – 1.92]	n/a	n/a
Number of women	9,955 (43.0)	102,518 (47.3)	102,518 (47.3)
Age			
Mean age at index date (years, SD)	59 (16.0)	61 (21.0)	61 (21.0)
18 - 49 years	5,022 (21.7)	46,551 (21.5)	46,909 (21.6)
50 - 59 years	6,563 (28.3)	44,634 (20.6)	45,132 (20.8)
60 - 69 years	7,061 (30.5)	56,830 (26.2)	56,760 (26.2)
70 - 79 years	3,721 (16.1)	47,376 (21.9)	47,025 (21.7)
80+ years	785 (3.4)	21,425 (9.9)	20,990 (9.7)
BMI			
Mean BMI at index date (kg/m^2, SD)	33 (6.6)	31 (6.6)	27 (5.1)
<20.0 kg/m^2	137 (0.6)	2,762 (1.3)	10,104 (4.7)
20.0 - 24.9 kg/m^2	2,008 (8.7)	27,053 (12.5)	64,626 (29.8)
25.0 - 29.9 kg/m^2	6,754 (29.2)	68,486 (31.6)	75,756 (34.9)
30.0 - 34.9 kg/m^2	7,029 (30.4)	60,195 (27.8)	30,681 (14.2)
≥35.0 kg/m^2	7,063 (30.5)	52,937 (24.4)	12,466 (5.7)
Missing	161 (0.7)	5,383 (2.5)	23,183 (10.7)
Smoking status			
Never	11,428 (49.4)	107,919 (49.8)	114,195 (52.7)
Current	4,677 (20.2)	43,055 (19.9)	45,187 (20.8)
Ex	7,015 (30.3)	64,745 (29.9)	51,686 (23.8)
Missing	32 (0.1)	1,097 (0.5)	5,748 (2.7)
Alcohol use			
No	6,511 (28.1)	62,695 (28.9)	39,364 (18.2)
Yes	15,898 (68.7)	142,011 (65.5)	152,802 (70.5)
Missing	743 (3.2)	12,110 (5.6)	24,650 (11.4)
Mean HbA1c (%, SD)	8.3 (1.8)	8.0 (1.8)	6.3 (1.2)
Missing	7,603 (32.8)	92,169 (42.5)	211,402 (97.5)
Falls (7 - 12 months before)	175 (0.8)	2,176 (1.0)	1,755 (0.8)
History of disease ever before			
Fracture	4,838 (20.9)	44,957 (20.7)	45,197 (20.8)
Hyperthyroidism	210 (0.9)	2,078 (1.0)	1,729 (0.8)
Hypothyroidism	1,794 (7.7)	17,123 (7.9)	11,280 (5.2)
COPD	1,006 (4.3)	11,922 (5.5)	9,898 (4.6)
Congestive heart failure	620 (2.7)	9,088 (4.2)	4,064 (1.9)
Cancer	4,793 (20.7)	47,244 (21.8)	50,047 (23.1)
Rheumatoid arthritis	357 (1.5)	3,741 (1.7)	3,406 (1.6)
Retinopathy	2,947 (12.7)	26,202 (12.1)	1,365 (0.6)
Secondary osteoporosis	1,501 (6.5)	19,447 (9.0)	7,866 (3.6)
Neuropathy	1,645 (7.1)	16,404 (7.6)	2,747 (1.3)

Table 2.2.1 (continued)

Characteristic	DPP4-I users[b] N=22,510	NIAD users[c] N=216,816	Controls[d] N=216,816
Drug use within six months before			
Metformine	20,428 (88.2)	179,104 (82.6)	n/a
Sulfonylurea derivatives	8,939 (38.6)	58,024 (26.8)	n/a
TZDs	4,749 (20.5)	22,561 (10.4)	n/a
Insulins	1,023 (4.4)	24,276 (11.2)	n/a
Oral glucocorticoids	1,090 (4.7)	12,512 (5.8)	8,602 (4.0)
Statins	15,790 (68.2)	118,531 (54.7)	45,338 (20.9)
Antiarrhythmics	362 (1.6)	3,453 (1.6)	2,904 (1.3)
Antidepressants	3,849 (16.6)	34,930 (16.1)	22,874 (10.5)
Anti-Parkinson	82 (0.4)	1,061 (0.5)	1,197 (0.6)
Antipsychotics	367 (1.6)	4,493 (2.1)	2,617 (1.2)
Anxiolytics	1,486 (6.4)	15,376 (7.1)	12,645 (5.8)
Hypnotics	1,010 (4.4)	10,661 (4.9)	9,050 (4.2)
Antihypertensives	14,895 (64.3)	125,397 (57.8)	70,861 (32.7)
Bisphosphonates	391 (1.7)	5,646 (2.6)	7,006 (3.2)
Raloxifene	16 (0.1)	303 (0.1)	172 (0.1)
Vitamin D	79 (0.3)	1,198 (0.6)	748 (0.3)
Calcium	646 (2.8)	8,399 (3.9)	9,545 (4.4)
Strontium ranelate	10 (0.0)	124 (0.1)	256 (0.1)
PTH/calcitonin	0 (-)	0 (-)	2 (0.0)
Hormone replacement therapy	158 (0.7)	904 (0.4)	1,618 (0.7)

BMI: body mass index; COPD: chronic obstructive pulmonary disease; DPP4-I: dipeptidyl peptidase 4 inhibitor; IQR: inter quartile range; n/a: not applicable; NIAD: non-insulin anti-diabetic drug; PTH: parathyroid hormone; SD: standard deviation; TZD: thiazolidinedione; secondary osteoporosis; hypogonadism or premature menopause (<45 year). [a] Data are number (%) of patients, unless stated otherwise. [b] DPP4-I users are patients who have at least 1 prescription of a DPP4-I during follow-up. [c] NIAD users are patients who have at least 1 NIAD prescription during follow-up. [d] Controls are patients who do not have a NIAD prescription during the entire study period.

Table 2.2.2 shows that the risk of fracture is similar when comparing the estimated hazard ratio of current DPP4-I use to that of the controls (adjusted hazard ratio (adj. HR) 0.89, 95% confidence interval (CI): 0.71-1.13). Recent DPP4-I users had a significantly increased risk of fracture compared to the controls, adj. HR 1.54 (95% CI: (1.08-2.18)). The adj. HR of past DPP4-I users was 1.01 (95% CI: 0.91-1.13) compared to the controls.

Current DPP4-I use was further stratified according to current exposure to insulin and TZDs because these drugs are known to increase fracture risk[4-6]. Current DPP4-I users who were not exposed to TZDs showed an adj. HR of 0.89 (95% CI: 0.79-1.02) as compared to controls. The adj. HR for current DPP4-I users who were also current TZD user was 1.77 (95% CI: 1.42-2.20). Current DPP4-I users who were not exposed to current insulin use showed an adj. HR of 0.99 (95% CI: 0.88-1.11), for current DPP4-I users exposed to insulin it was 1.36 (95% CI: 0.97-1.90), as compared to controls, Table 2.2.2.The risk of fracture among current DPP4-I users was comparable to the risk of other current NIAD users, Table 2.2.3. The adj. HR for current DPP4-I use was 1.03 (95% CI: 0.92-1.15). The adj. HR of recent DPP4-I use was significantly increased, adj. HR 1.49

(95% CI: 1.05-2.11), and the HR of past DPP4-I use was 0.86 (95% CI: 0.68-1.08) all compared to other NIAD users. The stratified analysis for the different outcome types, sex and age categories are shown in Table 2.2.3.

Table 2.2.2 Risk of fracture in DPP4-I users compared with controls, by type of fracture, age, and sex[a].

	No of fractures N=18,445	Fracture IR (/1000 PY)	Age/sex adjusted HR (95% CI)	Adjusted HR (95% CI)
No NIAD use	9,105	12.4	Reference	Reference
Past NIAD use	711	6.4	0.56 (0.52 - 0.61)*	0.55 (0.51 - 0.60)*[,c]
Current NIAD use	8,629	14.0	1.16 (1.12 - 1.19)*	1.04 (1.01 - 1.08)*[,c]
By type of NIAD[b]				
Past DPP4-I use	75	11.6	1.11 (0.99 - 1.23)	1.01 (0.91 - 1.13)[c]
Recent DPP4-I use	32	19.2	1.70 (1.20 - 2.42)*	1.54 (1.08 - 2.18)*[,c]
Current DPP4-I use	347	11.8	0.99 (0.79 - 1.25)	0.89 (0.71 – 1.13)[c]
By current TZD use				
Yes	83	19.7	1.95 (1.57 - 2.43)*	1.77 (1.42 - 2.20)*[,c]
No	264	10.4	0.97 (0.86 - 1.10)	0.89 (0.79 - 1.02)[c]
By current insulin use				
Yes	35	18.6	1.71 (1.22 - 2.39)*	1.36 (0.97 - 1.90)[c]
No	312	11.3	1.07 (0.95 - 1.19)	0.99 (0.88 - 1.11)[c]
By sex[d]				
Males	130	7.5	1.01 (0.85 - 1.21)	0.87 (0.72 - 1.04)[c]
Females	217	18.0	1.16 (1.02 - 1.33)*	1.11 (0.96 - 1.27)[c]
By age[e]				
<50 years	26	5.9	0.81 (0.55 - 1.20)	0.65 (0.43 - 0.97)*[,c]
50 - 59 years	73	9.8	1.20 (0.95 - 1.53)	1.06 (0.82 - 1.37)[c]
60 - 69 years	99	10.1	1.05 (0.86 - 1.29)	0.88 (0.71 - 1.09)[c]
70 - 79 years	103	16.7	1.25 (1.02 - 1.52)*	1.16 (0.95 - 1.42)[c]
80+ years	46	27.0	1.15 (0.85 - 1.54)	1.08 (0.80 - 1.45)[c]
By type of fracture				
Major Osteoporotic	160	5.3	1.16 (0.99 - 1.37)	1.10 (0.93 - 1.29)[f]
Hip fracture	37	1.2	1.15 (0.82 - 1.59)	1.10 (0.79 - 1.54)[g]
Radius Ulna	64	2.1	1.20 (0.94 - 1.55)	1.16 (0.89 - 1.50)[h]
Vertebral fracture	17	0.6	0.84 (0.52 - 1.36)	0.76 (0.46 - 1.24)[i]

CI: confidence interval; DPP4-I: dipeptidyl peptidase 4 inhibitor: HR: hazard ratio; IR: incidence rate; NIAD: non-insulin anti-diabetic drug; PY: person years; TZD: thiazolidinedione. Current NIAD use: most recent NIAD prescription within 90 days before start of an interval. Past NIAD use: most recent prescription over 90 days before start of an interval. Current DPP4-I use: most recent prescription within 90 days before start of an interval. Recent DPP4-I use: most recent prescription within 91–180 days before start of an interval. Past DPP4-I use: most recent prescription over 180 days before start of an interval. * Statistical significant, (P<0.05). [a] All models are statistically adjusted for glucagon-like peptide 1 receptor agonist use. [b] Other NIAD types not shown. [c] Adjusted for (j) and the use of statins, hypnotic/anxiolytic drugs, anti-osteoporotic drugs[k], anti-depressants, and anti-psychotics. [d] Model not statistically adjusted for gender. [e] Model not statistically adjusted for age. [f] Adjusted for (j) and history of chronic obstructive pulmonary disease, use of statins, alcohol, anti-depressants, anti-psychotics, hypnotic/anxiolytic drugs, vitamin d, and calcium. [g] Adjusted for (j) and the use of anti-psychotics and anti-depressants. [h] Adjusted for (j) and the use of hypnotics/anxiolytic drugs, anti-osteoporotic drugs[k] and anti-psychotics. [i] Adjusted for (j) and the use of anti-osteoporotic drugs[k], and anti-pshychotics. [j] Sex, age, bmi, smoking status, history of neuropathy, retinopathy and secondary osteoporosis. [k] Use of bisphosphonates, raloxifene, strontium ranelate or parathyroid hormone/calcitonin.

Table 2.2.3 Risk of fracture in DPP4-I users compared with NIAD users, by type of fracture, age, and sex[a].

	No of fractures N=9,340	Fracture IR (/1000 PY)	Age/sex adjusted HR (95% CI)	Adjusted HR (95% CI)
No DPP4-I use[b]	8,886	12.9	Reference	Reference
Past DPP4-I use	75	11.6	0.98 (0.70 - 1.12)	0.86 (0.68 - 1.08)[c]
Recent DPP4-I use	32	19.2	1.51 (1.07 - 2.14)*	1.49 (1.05 - 2.11)*[,c]
Current DPP4-I use	347	11.8	0.96 (0.86 - 1.07)	1.03 (0.92 - 1.15)[c]
By sex[d]				
Males	130	7.5	0.88 (0.74 - 1.05)	0.96 (0.80 - 1.14)[c]
Females	217	18.0	1.01 (0.88 - 1.16)	1.08 (0.94 - 1.24)[c]
By age[e]				
<50 years	26	5.9	0.68 (0.45 - 1.00)	0.73 (0.49 - 1.09)[c]
50 - 59 years	73	9.8	1.08 (0.85 - 1.38)	1.22 (0.95 - 1.56)[c]
60 - 69 years	99	10.1	0.95 (0.77 - 1.16)	1.02 (0.83 - 1.25)[c]
70 - 79 years	103	16.7	1.10 (0.90 - 1.35)	1.16 (0.95 - 1.42)[c]
80+ years	46	27.0	0.95 (0.70 - 1.27)	1.00 (0.74 - 1.34)[c]
By type of fracture				
Major osteoporotic	160	5.3	1.00 (0.85 - 1.17)	1.07 (0.91 - 1.25)[f]
Vertebral fracture	17	0.6	0.84 (0.51 - 1.37)	0.96 (0.58 - 1.57)[g]
Hip fracture	37	1.2	0.89 (0.64 - 1.24)	0.97 (0.69 - 1.35)[h]
Radius Ulna	64	2.1	1.14 (0.88 - 1.47)	1.19 (0.92 - 1.55)[i]

CI: confidence interval; DPP4-I: dipeptidyl peptidase 4 inhibitor; HR: hazard ratio; IR: incidence rate; PY: person years. Current DPP4-I use: most recent prescription within 90 days before start of an interval. Recent DPP4-I use: Most recent prescription within 91–180 days before start of an interval. Past DPP4-I use: most recent prescription more than 180 days before start of an interval. * Statistically significant, (P<0.05). [a] All models are statistically adjusted for glucagon-like peptide 1 receptor agonist use. [b] NIAD past use not shown. [c] Adjusted for (j) and the use of statins, hypnotic/anxiolytic drugs, anti-osteoporotic drugs[k], anti-depressants, and anti-psychotics. [d] Model not statistically adjusted for gender. [e] Model not statistically adjusted for age. [f] Adjusted for (j) and history of chronic obstructive pulmonary disease, use of statins, alcohol, anti-depressants, anti-psychotics, hypnotic/anxiolytic drugs, vitamin d, and calcium. [g] Adjusted for (j) and the use of anti-psychotics and anti-depressants. [h] Adjusted for (j) and the use of hypnotics/anxiolytic drugs, anti-osteoporotic drugs[k] and anti-psychotics. [i] Adjusted for (j) and the use of anti-osteoporotic drugs[k], and anti-pshychotics. [j] sex, age, body mass index, smoking status, history of neuropathy, retinopathy and secondary osteoporosis, glycosylated hemoglobin type A1c and the use of insulin and thiazolidinediones. [k] Use of bisphosphonates, raloxifene, strontium ranelate or parathyroid hormone /calcitonin.

Table 2.2.4 shows that the HRs were comparable for the different stratified groups of current DPP4-I use (i.e. by average daily dose and cumulative daily dose). The adj. HR for the highest average daily dose was 1.34 (95% CI: 0.83-2.17) and the adj. HR for the highest cumulative dose was 1.02 (95% CI: 0.82-1.26). The adj. HR for the low MPR (MPR <80%) was 1.04 (95% CI: 0.93-1.17) and the adj. HR of the high group (MPR ≥80%) was 0.86 (95% CI: 0.59-1.24). After correction for TZD users the adj. HR for current DPP4-I users was 1.04 (95% CI: 0.93-1.16).

Table 2.2.4 Risk of fracture in current DPP4-I users stratified by average and cumulative dose[a].

	No of fractures N=9,340	Fracture IR (/1000 PY)	Age/sex adjusted HR (95% CI)	Adjusted HR (95% CI)[c]
No DPP4-I use[b]	8,886	12.9	Reference	Reference
Current DPP4-I use	347	11.8	0.96 (0.86 - 1.07)	1.03 (0.92 - 1.15)
By average dose exposure				
0 - 49 mg sitagliptin equivalents	172	11.0	0.91 (0.78 - 1.06)	0.97 (0.84 - 1.13)
50 - 99 mg sitagliptin equivalents	119	11.6	0.93 (0.77 - 1.11)	1.00 (0.83 - 1.20)
≥100 mg sitagliptin equivalents	17	16.1	1.29 (0.80 - 2.07)	1.34 (0.83 - 2.17)
No dose	39	15.4	1.26 (0.92 - 1.73)	1.34 (0.98 - 1.84)
By cumulative dose exposure				
0 - 18.2 g sitagliptin equivalents	128	12.8	1.04 (0.87 - 1.24)	1.10 (0.92 - 1.31)
18.3 - 36.5 g sitagliptin equivalents	75	10.0	0.82 (0.65 - 1.03)	0.89 (0.71 - 1.12)
36.6 - 54.7 g sitagliptin equivalents	57	12.5	1.02 (0.79 - 1.33)	1.11 (0.86 - 1.44)
>54.7 g sitagliptin equivalents	87	11.6	0.95 (0.77 - 1.17)	1.02 (0.82 - 1.26)

CI: confidence interval; HR: hazard ratio; IR: incidence rate; PY: person years; DPP4-I: dipeptidyl peptidase 4 inhibitor. [a] Also corrected for glucagon-like peptide 1 receptor agonist use. [b] NIAD past use, DPP4-I recent and DPP4-I past use not shown. [c] Adjusted for sex, age, body mass index, smoking status, glycosylated hemoglobin type A1c, history of secondary osteoporosis, retinopathy and neuropathy, use of statins, hypnotic/anxiolytic drugs, anti-osteoporotic drugs[d], anti-depressants, anti-psychotics, insulin and thiazolidinediones. [d] Use of bisphosphonates, raloxifene, strontium ranelate or parathyroid hormone /calcitonin.

Discussion

The present study showed that there was no significant association with current DPP4-I use and fracture risk as compared to controls. Additionally, we showed that there was no association with DPP4-I use and fracture risk as compared to other NIAD users. Moreover, when stratifying current DPP4-I use by cumulative and average daily dose there was no association with fracture risk as compared to other NIAD users. The risk of hip, major osteoporotic and vertebral fracture was also not different between the DPP4-I and the NIAD users.

Our results are not in line with the main result found in a meta-analysis of RCTs by Monami et al.[14]. That study showed a 40% reduction of fracture risk for DPP4-I users compared to patients taking other anti-hyperglycaemic drugs or placebo[14]. Differences between the meta-analysis and our study could be explained by several factors. First, in the meta-analysis fractures were not routinely collected, they were only considered when reported as a severe adverse event. The present study used CPRD data which, for hip fracture has shown to be highly valid with over 90% confirmed routinely collected diagnoses[16]. Second, the mean duration of follow-up was rather short (35 weeks). It might be expected that due to the short follow-up and the low number of fractures the potential beneficial effect of DPP4-I on fracture risk was attenuated.

The results of our study are in keeping with a recently published large RCT (n=16,492) that compared saxagliptin, a DPP4-I, to placebo[19]. That study showed that

there was no significant difference in the number of bone fractures between saxagliptin and placebo users[19]. The results of the present study are in line with, to our knowledge, the only two clinical trials that investigated the effect of incretin (i.e. exenatide and vildagliptin) treatment on markers of bone remodelling and calcium homeostasis[15,20]. Both studies did not show an effect on calcium homeostasis and other markers reflecting bone resorption after one year and 44 weeks of treatment, respectively, which suggests that the risk of fracture is not altered after treatment with incretin mimetics.

The observation of a significantly 1.5-fold increased risk of fracture for recent DPP4-I users compared to controls and to other NIAD users was unexpected. It is difficult to explain this increased risk of fracture, which was only found in the recent user group, by an plausible underlying mechanism and we cannot rule out the play of chance. Users of both TZDs and DPP4-Is had a 1.8-fold increased fracture risk compared to controls without diabetes. TZDs have shown to increase fracture risk[4-6] and this effect was still present in our study despite the potential protective effect of the DPP4-Is. Different mechanisms have been hypothesized by which GLP-1 might have a beneficial effect on bone. The enzyme dipetidyl peptidase 4 is involved in the degradation of GLP-1. DPP4-Is are able to inhibit this process and thereby prolonging the half-life of GLP-1. In vitro research has shown that different cell types have a GLP-1 receptor, including osteoblasts and osteocytes[12,13]. Besides, it has been shown that binding of GLP-1 to osteoblasts resulted in an increase of calcitonin which ultimately might lead to decreased bone resorption[10]. Another study showed that after binding of a GLP1-RA, Exendin-4, to osteocytes, the production of sclerostin was decreased[21]. This might in the end result in increased bone formation. Thyroid C cells express a GLP-1 receptor as well[22]. Another in vitro study showed that binding of GLP-1 resulted in increased secretion of calcitonin which could inhibit bone resorption[22]. However, more research is needed to elucidate whether DPP4-Is indeed are able, via the prolonging of the half-life of GLP-1, to increase bone formation and inhibit bone resorption and whether this effect is sufficient to reduce fracture risk in humans.

Our study had several strengths. We were able to statistically adjust in a time-dependent way for several potentially important confounders, including, age, HbA1c, different comorbidities, such as retinopathy and neuropathy, and drug use. Besides, we had information at baseline about gender, smoking, and BMI for almost all patients. By adjusting our analyses for neuropathy, retinopathy and HbA1c, we tried to adjust for the disease severity, which is a potential confounder. Additionally, because CPRD data is collected prospectively recall bias was probably not an issue in the present study. Finally, a substantial amount of data representing the general population of the United Kingdom was available. Our main limitation was the mean duration of actual DPP4-I use (time from the first DPP4-I prescription until the last DPP4-I prescription) which was, despite the mean duration of follow-up of 4.0 years, 1.3 years. Drugs used for the treatment of osteoporosis, such as bisphosphonates, have shown a diminishing effect

on the risk of fracture after 1-1.5 years of use[23,24]. This suggests that our follow-up time might be too short to have enough power to detect an effect of the use of DPP4-Is on the risk of fracture.

It is possible that there is still some residual confounding present. For instance, we were not able to correct for the amount of exercise. Exercise has been shown to reduce the risk of fracture[25]. DPP4-I users might perform less exercise compared to non-DPP4-I users, which could lead to an overestimation of our effect. Another potential source of bias is a selective prescribing of DPP4-Is to patients who are known to use more than the average amount of alcohol. Alcohol use has been associated with hypoglycaemic events[26] and with an increased risk of fracture[27]. DPP4-Is have been associated with a lower risk of hypoglycaemic events compared to, for example, sulfonylurea derivatives[28]. The selective prescribing could therefore mask the potential beneficial effect of DPP4-Is on fracture risk. Although, we statistically adjusted our main analysis for alcohol use (yes, no or missing), this did not change our results (data not shown), but underreporting of the amount of alcohol patients use might be an issue.

In summary, we showed that DPP4-I use is not associated with fracture risk compared to controls and to other NIAD users. However, our duration of DPP4-I use was probably too short to show an association with fracture risk. Therefore, a longer duration of DPP4-I use is needed to further investigate the potential protective effect of DPP4-Is on fracture risk.

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Chapter 2.3

Use of dipeptidyl peptidase 4 inhibitors and fracture risk compared to use of other anti-hyperglycaemic drugs

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Abstract

Introduction

Dipeptidyl peptidase 4 inhibitors (DPP4-Is) are a new class of anti-hyperglycaemic drugs which might have a potential beneficial effect on bone metabolism. Data on the effect of DPP4-I use and fracture risk is limited and conflicting. The aim of the present study was to investigate the association between use of DPP4-Is and fracture risk.

Methods

A case-control study was conducted using data from the Danish National Health Service. Cases were those who sustained a fracture and controls were those without a fracture during the study period (2007-2011), all aged 18 years and older. Conditional logistic regression estimated the odds ratios of fracture with current use of DPP4-Is. Analyses were adjusted for comorbidities and recent drug use.

Results

Among the cases there were 6,993 current non-insulin anti-diabetic drug (NIAD) users (excluding incretin users) and 643 DPP4-I users. There were 7,209 NIAD users (excluding incretin users) among the controls and 707 DPP4-I users. Current DPP4-I use was not associated with risk of any fracture (adjusted [adj.] OR: 0.97, 95% CI: 0.79-1.18) or major osteoporotic fracture (adj. OR: 0.96, 95% CI: 0.72-1.28). Stratification of current DPP4-I use to cumulative and average daily dose did not show an association.

Conclusion

In a population-based case-control study we identified that short-term use of DPP4-I was not associated with fracture risk as compared to users of other anti-hyperglycaemic drugs. Additionally, results suggest that increasing daily dose and cumulative DPP4-I exposure were not associated with fracture risk. However, more research is needed to assess the effect of long-term DPP4-I use on the risk of fracture.

Introduction

Individuals with type 2 diabetes are at an excess risk of osteoporotic fractures[1]. Although the aetiology of this increased risk is largely unknown, it has been hypothesized that this might be due to the underlying disease and/or to the drugs used in the treatment of type 2 diabetes[2,3]. For instance, thiazolidinediones have been associated with an increased fracture risk as compared to use of other anti-hyperglycaemic drugs[4-6]. Moreover, epidemiological studies have shown that insulin use is associated with an elevated risk of fracture[7], whereas metformin has been associated with a decreased risk of fracture[8].

Dipeptidyl peptidase 4 inhibitors (DPP4-Is), such as sitagliptin, vildagliptin and saxagliptin, are a new class of anti-hyperglycaemic drugs, which may positively affect bone metabolism[3] and decrease fracture risk. DPP4-Is inhibit the degradation process of incretin hormones, such as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), which are secreted shortly after food ingestion[3]. Thus, DPP4-Is prolong the half-life time of the incretin hormones. In vitro research has shown that there are GLP-1 receptors present on osteocytes[9] and immature osteoblast cells[10]. Activation of this receptor leads to an increased bone formation and an inhibition of bone resorption[5,11], which might result in a decreased fracture risk.

Prior evidence on the association between DPP4-I use and risk of fracture are conflicting and limited mostly to clinical trial data. A meta-analysis of clinical trials showed a 40% reduction in fracture risk associated with DPP4-I use[12]. However, the study had several limitations: the total number of fractures was small, fracture was not a primary endpoint and fractures were not routinely collected, there were heterogeneous comparison groups and it might be that relatively healthy patients were included in the trials. A large clinical trial (n=16,492, with a median follow-up time of 2.1 years), comparing saxagliptin to placebo showed a relative risk of fracture around 1[13]. An observational study investigating the effect of DPP4-I use on fracture risk showed a hazard ratio of 1.03 (95% confidence interval (CI): 0.92-1.15) as compared to use of other anti-hyperglycaemic drugs[14].

With limited available data, the aim of the present study was to examine the association between use of DPP4-Is and fracture risk compared to use of other anti-hyperglycaemic drugs in a population-based case-control study.

Methods

Data source

In Denmark, the extensive nature of health registries, covering all contacts to the health sector, offers the possibility for large population-based studies on the occurrence of fractures[15]. Using the unique 10-digit civil registry number that is assigned to all Danish

citizens shortly after birth, a complete hospital discharge and prescription history can be established for each individual, and permits valid linkage between population-based registries[16]. Data on all changes in vital status, including change of address and date of death for the entire Danish population are registered since 1968 in the Civil Registration System. The Danish National Hospital Discharge Registes[17] was founded in 1977 and covers all inpatients contacts from 1977 to 1994. Additionally, all outpatient visits to hospitals, outpatient clinics, and emergency rooms have been collected since 1995.

In Denmark, pharmacies are equipped with a computerised accounting system through which data are sent directly to a Register of Medicinal Product Statistics (i.e. a prescription database) at the Danish Medicines Agency with key information on prescriptions for refundable drugs. The prescription database includes information on patient's civil registry number, the type and amount of drug prescribed according to the Anatomical Therapeutic Chemical (ATC) classification system and the date when the prescription was filled. The database was started on January 1, 1996 and updated hereafter.

The Danish population constituted approximately 5.2 million individuals in 1995 and 5.5 million in 2011. The study was subject to control by the National Board of Health and the Danish Data Protection Agency.

Study design

We conducted a population-based case-control study. Cases were all subjects, both genders and aged 18 years and older, who sustained a fracture (International Classification of Diseases and Related Health Problems [ICD]-10 codes: S02, S12, S22, S32, S42, S52, S62, S72, S82, S92, T02, T08, T10, and T12) between 9 May 2007 (the first ever prescription of a DPP4-I in Denmark) and 31 December 2011. Information on fractures was on based in- and outpatient hospital records. Controls were all subjects, both genders and aged 18 years and older, who did not sustain a fracture during the study period. We randomly selected one control for each case, matched by gender and year of birth by means of the incidence-density sampling technique[18]. The date of the first fracture was used as index date and controls were assigned the index date of their matched case. The following categories were used to further classify fractures: hip (S72.0-S72.2), radius/ulna (S52) and vertebrae (S12, S22.0-S22.1, S32.0-S32.2, S32.7, S32.8, T08). A major osteoporotic fracture was defined as a fracture of the hip, radius/ulna, vertebrae or humerus (S42.2-S42.4) according to the WHO definition[19].

Exposure of interest

We explored all drugs bought during the observation period available in the database. The dose of the drug bought during the observation period was expressed as defined daily dose (DDD)[20]. ATC code A10B was used to determine exposure to non-insulin anti-

diabetic drugs (NIAD). Based on the time of the most recent prescription before the index date, patients were classified as current (1-91 days) or past (over 91 days) NIAD user. Current NIAD users were divided into two mutually exclusive categories: never (during the study period) incretin users and incretin users. As diabetes itself might act as a confounder[2] we used never incretin users (i.e. patients using non-insulin anti-diabetic drugs excluding incretins) as the reference category in our analysis. Incretin use was further divided into glucagon-like peptide 1 receptor agonists (GLP1-RAs) users and DPP4-I users. DPP4-I exposure was determined by use of ATC codes A10BD07-A10BD13 and A10BH. DPP4-I users were classified as current (1-91 days), recent (92-182), past (183-365 days) or distant (over 365 days) users based on the time since the most recent prescription before the index date.

The DDDs were used to estimate the cumulative dose of DPP4-Is for each current DPP4-I user, expressed as sitagliptin equivalents. The average daily dose was estimated by dividing the cumulative exposure by the treatment time (time between the first DPP4-I prescription and the index date).

Potential confounders

We considered the following potential confounders ever before the index date: a history of chronic obstructive pulmonary disease, previous fracture, rheumatoid arthritis, hypothyroidism, hyperthyroidism, cancer, alcoholism, retinopathy, secondary osteoporosis (diabetes type 1, hypogonadism or premature menopause) and congestive heart failure. The potential confounders were determined by use of the National Hospital Register based on ICD10 and ICD8 codes. Other potential confounders included a prescription in the six months before the index date of the following drugs: GLP1-RAs, oral glucocorticoids[21], cholesterol lowering drugs, antidepressants[22], anxiolytics, hypnotics[23], antipsychotics, anti-Parkinson drugs[24], antihypertensives (beta-blockers, thiazide diuretics, renin angiotensin aldosterone system inhibitors, calcium channel blockers and loop diuretics), and antiarrhythmics. The prescription database was used to explore the presence of a prescription of the above-mentioned drugs.

Statistical analysis

Conditional logistic regression was used to estimate the association between the use of DPP4-Is versus use of other anti-hyperglycaemic drugs and risk of fracture, using SAS 9.3 software. Analyses were stratified by age, gender, type of fracture and for current DPP4-I use also by average daily and cumulative exposure. As a sensitivity analysis the main analyses was also adjusted for current metformin use, because metformin use has been associated with a decreased risk of fracture[8]. Final regression models were determined by stepwise backward elimination using a significance level of 0.05. All results are presented as odds ratios (ORs) with corresponding 95% CIs.

Results

We identified 229,145 cases and the same number of controls. In both cases and controls, the mean age was 55 years old and 56% were females. Baseline characteristics are shown in Table 2.3.1. There were 643 (0.3%) cases and 707 (0.3%) controls that were DPP4-I users (current, recent, past or distant). The mean duration of DPP4-I use (time from first prescription till index fracture date) for current DPP4-I use was 47 weeks for both cases and controls.

Table 2.3.1 Baseline characteristics[a].

Characteristic	Cases (N=229,145)	Controls (N=229,145)
Women	127,449 (55.6)	127,449 (55.6)
Mean age at index date (years, SD)	55 (20.6)	55 (20.6)
18 - 49 years	90,598 (39.5)	90,607 (39.5)
50 - 59 years	37,247 (16.3)	37,191 (16.2)
60 - 69 years	38,751 (16.9)	38,805 (16.9)
70 - 79 years	28,950 (12.6)	28,931 (12.6)
80+ years	33,599 (14.7)	33,611 (14.7)
History of comorbidities		
Type 1 diabetes mellitus	2,113 (0.9)	1,419 (0.6)
Alcoholism	11,147 (4.9)	4,824 (2.1)
Fracture	46,446 (20.2)	15,418 (6.7)
Hyperthyroidism	3,715 (1.6)	3,688 (1.6)
Hypothyroidism	2,887 (1.3)	2,496 (1.1)
COPD	10,812 (4.7)	7,418 (3.2)
Congestive heart failure	7,141 (3.1)	5,424 (2.4)
Cancer	21,893 (9.6)	18,486 (8.1)
Rheumatoid arthritis	3,912 (1.7)	2,795 (1.2)
Retinopathy	3,105 (1.4)	2,314 (1.0)
Neuropathy	7,915 (3.5)	6,093 (2.7)
Secondary osteoporosis	5,284 (2.3)	3,352 (1.5)
Drug use within six months before index date		
Diabetic Medication	8,541 (3.7)	8,676 (3.8)
Biguanides	6,223 (2.7)	6,678 (2.9)
Sulphonylurea derivatives	3,900 (1.7)	3,809 (1.7)
Thiadolidinediones	262 (0.1)	183 (0.1)
Glinides	87 (0.0)	83 (0.0)
GLP1-RAs	255 (0.1)	220 (0.1)
DPP4-Is	643 (0.3)	707 (0.3)
Acarbose	42 (0.0)	28 (0.0)
Insulins	4,900 (2.1)	3,261 (1.4)
Short acting	2,046 (0.9)	1,139 (0.5)
Intermediate acting	2,049 (0.9)	1,411 (0.6)
Long acting	1,444 (0.6)	869 (0.4)
Combinations	1,679 (0.7)	1,200 (0.5)

Table 2.3.1 (continued)

Characteristic	Cases (N=229,145)	Controls (N=229,145)
Statins	31,874 (13.9)	32,064 (14.0)
Antiarrhythmics	818 (0.4)	522 (0.2)
Beta-blockers	23,281 (10.2)	23,592 (10.3)
Thiazide diuretics	20,425 (8.9)	21,547 (9.4)
RAAS inhibitors	37,555 (16.4)	39,379 (17.2)
Calcium channel blockers	22,942 (10.0)	22,816 (10.0)
Loop diuretics	16,905 (7.4)	12,766 (5.6)
Antidepressants	33,644 (14.7)	20,338 (8.9)
Anti-Parkinson drugs	3,174 (1.4)	1,814 (0.8)
Antipsychotics	8,032 (3.5)	4,867 (2.1)
Anxiolytics	14,668 (6.4)	10,431 (4.6)
Hypnotics	19,137 (8.4)	14,332 (6.3)
Glucocorticoids	9,390 (4.1)	6,858 (3.0)
Bisphosphonates	7,371 (3.2)	4,913 (2.1)
Raloxifene	214 (0.1)	148 (0.1)
Vitamin D	211 (0.1)	169 (0.1)
Calcium	1,885 (0.8)	1,375 (0.6)
Strontium ranelate	194 (0.1)	101 (0.0)
Parathyroid hormone	130 (0.1)	70 (0.0)
Calcitonin	2 (0.0)	1 (0.0)
Hormone replacement therapy	11,912 (5.2)	13,955 (6.1)
Beta2-agonists	10,436 (4.6)	8,130 (3.6)
Inhaled anticholinergics	4,569 (2.0)	3,277 (1.4)
Inhaled corticosteroids	5,265 (2.3)	4,657 (2.0)

COPD: chronic obstructive pulmonary disease; DPP4-I: dipeptidyl peptidase 4 inhibitor; GLP1-RA: glucagon-like peptide 1 receptor agonist; RAAS: renin angiotensin aldosterone system; SD: standard deviation. [a] Data are number (%) of patients, unless stated otherwise.

We identified, 6,993 (3.1%) cases and 7,209 (3.1%) controls with current NIAD users (excluding incretin users), Table 2.3.2. Never NIAD use was associated with a slightly increased risk of fracture, adjusted (adj.) OR 1.10 (95% CI: 1.06-1.14) as well as past NIAD use, adj. OR 1.12 (95% CI: 1.05-1.20). The risk of any fracture was not associated with current DPP4-I use adj. OR 0.97 (95% CI: 0.79-1.18), whereas recent use showed a significantly decreased risk of fracture, adj. OR 0.76 (95% CI: 0.59-0.97). The risk associated with past and distant DPP4-I use was not significantly decreased: adj. OR 0.96 (95% CI: 0.77-1.20), and adj. OR 0.95 (95% CI: 0.72-1.25), respectively. There was no decreased risk of fracture after stratification by sex and age, Table 2.3.2.

Current DPP4-I use was not associated with major osteoporotic fracture risk, adj. OR 0.96 (95% CI: 0.72-1.28), Table 2.3.3. Recent DPP4-I use showed a significantly decreased risk of major osteoporotic fracture adj. OR 0.70 (95% CI: 0.49-1.00), while past and distant DPP4-I use were not associated with a decrease in risk adj. OR 0.95 (95% CI: 0.71-1.29; adj. OR 0.94 (95% CI: 0.63-1.38, respectively). DPP4-I use (current, recent, past or distant) was not associated with risk of hip fracture or radius/ulna fracture. Current DPP4-I use was not associated with vertebral fracture risk whereas

recent DPP4-I use resulted in a decreased risk of vertebral fracture: adj. OR 0.27 (95% CI: 0.08-0.90). Past and distant use was not associated with a decreased risk of vertebral fracture: adj. OR 2.34 (95% CI: 0.87-6.32), adj. OR 1.01 (95% CI: 0.25-4.12), respectively. Stratification by gender did not result in a reduced fracture risk for women or men (data not shown).

Stratification of current DPP4-I use by cumulative and average daily dose did not show a significantly decreased risk of any fracture, Table 2.3.4. The adjusted OR for the highest cumulative dose was 1.08 (95% CI: 0.76-1.54) and for highest average daily dose was 0.83 (95% CI: 0.55-1.26). Stratification of current DPP4-I use by cumulative and average daily dose for the other fracture types did not result in a decreased risk of major osteoporotic, Table 2.3.4, hip, vertebral or radius/ulna fracture (data not shown).

Adjusting the main analysis for current metformin use did not substantially change the results. Adjusted OR with current DPP4-I use was 0.94 (95% CI: 0.77-1.15), recent DPP4-I use was 0.74 (95% CI: 0.58-0.95), past DPP4-I use was 0.93 (95% CI: 0.74-1.16) and distant DPP4-I use was 0.92 (95% CI: 0.70-1.22).

Discussion

The present study showed that current DPP4-I use was not associated with any fracture risk as compared to current use of other anti-hyperglycaemic drugs. We further identified that the risk of other fractures was not associated with current DPP4-I use. Stratification of current DPP4-I use to cumulative and average daily dose did not show an association with fracture risk. However, recent DPP4-I use was associated with a significantly decreased risk of any fracture.

Our results are in agreement with a large clinical trial (n=16,492, median follow-up 2.1 years) that compared saxagliptin (DPP4-I) to placebo and showed a non-significant relative fracture risk around 1.0[13]. However, fractures were not the primary endpoint. The results are also in keeping with a cohort study using data from the Clinical Practice Research Datalink (CPRD) reporting that fracture risk was not associated with DPP4-I use as compared to use of other anti-hyperglycaemic drugs[14]. Similarly, two meta-analyses of clinical trials on the effect of GLP1-RAs on fracture risk showed no association between GLP1-RAs and fracture risk[25,26]. As well as a retrospective cohort study comparing use of GLP1-RAs to use of other anti-hyperglycaemic drugs[27]. Although GLP1-RAs intervene on a different molecule than DPP4-Is, the final effect on bone metabolism might be similar[3]. Moreover, our results are supported by the results of a clinical trial investigating the effect of the DPP4-I vildagliptin on a bone resorption marker[28]. Compared to placebo, 44 weeks of treatment with vildagliptin was not associated with a decrease in postprandial markers of resorption[29], which indirectly supports our findings.

Table 2.3.2 Use of DPP4-I and risk of any fracture.

Exposure	No. of cases (N=229,145)[a]	No. of controls (N=229,145)[a]	Crude OR (95% CI)	Adjusted OR (95% CI)[b]
Never NIAD use	217,623	218,194	1.03 (0.99 - 1.06)	1.10 (1.06 - 1.14)*
Past NIAD use	3,631	2,815	1.33 (1.25 - 1.41)*	1.12 (1.05 - 1.20)*
Current NIAD use excluding incretin use	6,993	7,209	Reference	Reference
Distant DPP4-I use (>365 days before the index date)	112	121	0.95 (0.74 - 1.24)	0.95 (0.72 - 1.25)
Past DPP4-I use (183 - 365 days before index date)	181	186	1.00 (0.81 - 1.23)	0.96 (0.77 - 1.20)
Recent DPP4-I use (92 - 182 days before the index date)	131	168	0.80 (0.64 - 1.01)	0.76 (0.59 - 0.97)*
Current DPP4-I use (1 - 91 days before the index date)	219	232	0.97 (0.81 - 1.17)	0.97 (0.79 - 1.18)
By sex				
Males	103	94	1.10 (0.83 - 1.46)	1.14 (0.84 - 1.54)
Females	116	138	0.88 (0.69 - 1.13)	0.86 (0.66 - 1.12)
By age at index date				
<50 years	17	14	1.19 (0.58 - 2.47)	1.16 (0.54 - 2.50)
50 - 59 years	50	51	1.07 (0.71 - 1.61)	1.06 (0.68 - 1.64)
60 - 69 years	64	65	0.97 (0.68 - 1.39)	0.97 (0.67 - 1.42)
70 - 79 years	49	58	0.83 (0.56 - 1.22)	0.89 (0.59 - 1.36)
80+ years	39	44	0.96 (0.62 - 1.48)	0.92 (0.58 - 1.44)

CI: confidence interval, DPP4-I: dipeptidyl peptidase 4 inhibitor; GLP1-RA: glucagon-like peptide 1 receptor agonist; NIAD: non-insulin anti-diabetic drug; OR: odds ratio. Never NIAD use: No NIAD prescription before the index date. Past NIAD use: Most recent NIAD prescription over 91 days before index date. Current NIAD use: Most recent NIAD prescription within 91 days before index date. * Statistically significant, (P<0.05). [a] The numbers do not sum to the total number of fractures since GLP1-RA exposure is not shown. [b] Adjusted for history of cancer, chronic obstructive pulmonary disease, fracture, alcoholism, rheumatoid arthritis, secondary osteoporosis, hyperthyroidism, retinopathy, neuropathy, heart failure and use of GLP1-RA, glucocorticoids, statins, anxiolytics, hypnotics, antidepressants, antipsychotics, anti-Parkinson drugs, beta-blockers, thiazide diuretics, renin angiotensin aldosterone system inhibitors, loop diuretics and antiarrhythmics.

Table 2.3.3 Use of DPP4-I and risk fracture at different skeletal sites.

Fracture sites	No. of cases[a]	No. of controls[a]	Crude OR (95% CI)	Adjusted OR (95% CI)
Major osteoporotic fracture	N=96,774	N=96,774	-	-
Never NIAD use	90,297	90,677	1.02 (0.97 - 1.07)	1.07[b] (1.02 - 1.13)
Current NIAD use excl. incretin use	3,957	4,058	Reference	Reference
Distant DPP4-I use (>365 days before the index date)	59	66	0.91 (0.64 - 1.30)	0.94[b] (0.63 - 1.38)
Past DPP4-I use (183 - 365 days before index date)	96	106	0.92 (0.70 - 1.22)	0.95[b] (0.71 - 1.29)
Recent DPP4-I use (92 - 182 days before the index date)	63	90	0.71 (0.52 - 0.99)*	0.70[b] (0.49 - 1.00)*
Current DPP4-I use (1 - 91 days before the index date)	110	115	0.98 (0.75 - 1.28)	0.96[b] (0.72 - 1.28)
Hip	N=24,328	N=24,328	-	-
Never NIAD use	21,923	22,273	0.94 (0.87 - 1.01)	1.00[c] (0.92 - 1.09)
Current NIAD use excl. incretin use	1,454	1,388	Reference	Reference
Distant DPP4-I use (>365 days before the index date)	17	15	1.10 (0.55 - 2.21)	0.95[c] (0.42 - 2.15)
Past DPP4-I use (183 - 365 days before index date)	25	28	0.85 (0.49 - 1.46)	0.80[c] (0.44 - 1.47)
Recent DPP4-I use (92 - 182 days before the index date)	17	24	0.67 (0.36 - 1.26)	0.76[c] (0.38 - 1.51)
Current DPP4-I use (1 - 91 days before the index date)	31	23	1.28 (0.74 - 2.20)	1.41[c] (0.78 - 2.52)
Radius/ulna	N=47,905	N=47,905	-	-
Never NIAD use	45,848	45,423	1.29 (1.19 - 1.39)	1.29[d] (1.19 - 1.40)*
Current NIAD use excl. incretin use	1,273	1,617	Reference	Reference
Distant DPP4-I use (>365 days before the index date)	19	35	0.69 (0.39 - 1.20)	0.64[d] (0.35 - 1.17)
Past DPP4-I use (183 - 365 days before index date)	35	52	0.85 (0.55 - 1.31)	0.88[d] (0.56 - 1.38)
Recent DPP4-I use (92 - 182 days before the index date)	28	43	0.83 (0.51 - 1.34)	0.76[d] (0.46 - 1.28)
Current DPP4-I use (1 - 91 days before the index date)	44	60	0.93 (0.63 - 1.38)	0.84[d] (0.55 - 1.27)
Vertebral	N=9,004	N=9,004	-	-
Never NIAD use	8,396	8,450	1.01 (0.87 - 1.17)	1.11[e] (0.93 - 1.32)
Current NIAD use excl. incretin use	364	369	Reference	Reference
Distant DPP4-I use (>365 days before the index date)	4	8	0.52 (0.16 - 1.73)	1.01[e] (0.25 - 4.12)
Past DPP4-I use (183 - 365 days before index date)	14	8	1.90 (0.75 - 4.82)	2.34[e] (0.87 - 6.32)
Recent DPP4-I use (92 - 182 days before the index date)	5	12	0.42 (0.15 - 1.20)	0.27[e] (0.08 - 0.90)*
Current DPP4-I use (1 - 91 days before the index date)	10	8	1.31 (0.51 - 3.36)	1.51[e] (0.54 - 4.23)

CI: confidence interval; COPD: chronic obstructive pulmonary disease; DPP4-I: dipeptidyl peptidase 4 inhibitor; GLP1-RA: glucagon-like peptide 1 receptor agonist; NIAD: non-insulin anti-diabetic drug; OR: odds ratio; RAAS: renin angiotensin aldosterone system. Never NIAD use: No NIAD prescription before the index date. Current NIAD use: Most recent NIAD prescription within 91 days before index date. * Statistically significant, (P<0.05). [a] The numbers do not sum to the total number of fractures since past NIAD use and GLP1-RA exposure are not shown. [b] Adjusted for (f) and history of retinopathy, heart failure, COPD and use of glucocorticoids, statins, anxiolytics, antipsychotics, beta-blockers, thiazide diuretics, RAAS inhibitors and antiarrhythmics. [c] Adjusted for (f) and history of retinopathy, heart failure, COPD and use of statins, anxiolytics, RAAS inhibitors, antiarrhythmics, glucocorticoids and antipsychotics. [d] Adjusted for (f) and use of beta-blockers, thiazide diuretics, RAAS inhibitors and calcium channel inhibitors. [e] Adjusted for (f) and history of COPD and use of glucocorticoids, anxiolytics, RAAS inhibitors and antipsychotics. [f] History of cancer, alcoholism, fracture, rheumatoid arthritis and secondary osteoporosis and use of GLP1-RAs, antidepressants, anti-Parkinson drugs, loop diuretics, and hypnotics.

Table 2.3.4 Current use of DPP4-I stratified by cumulative and average daily dose and risk of any and major osteoporotic fracture.

	Any Fracture			Major Osteoporotic Fracture		
Exposure	No. of cases (N=229,145)[a]	No. of controls (N=229,145)[a]	Adjusted OR (95% CI)[b]	No. of cases (N=96,774)[a]	No. of controls (N=96,774)[a]	Adjusted OR (95% CI)[c]
Current NIAD use excl. incretin use	6,993	7,209	Reference	3,957	4,058	Reference
Current DPP4-I use	219	232	0.97 (0.79 - 1.18)	110	115	0.96 (0.72 - 1.28)
By cumulative exposure[d]						
≤9.1 g	58	64	0.82 (0.56 - 1.20)	34	35	0.87 (0.52 - 1.46)
9.2 - 18.2 g	46	49	0.99 (0.65 - 1.52)	20	28	0.79 (0.43 - 1.46)
18.3 - 36.5 g	44	50	0.97 (0.63 - 1.49)	19	17	1.26 (0.63 - 2.54)
>36.5 g	71	69	1.08 (0.76 - 1.54)	37	35	1.04 (0.64 - 1.71)
By average daily dose[d]						
<100 mg/day	44	59	1.17 (0.75 - 1.81)	22	32	1.09 (0.56 - 2.11)
100 - 149 mg/day	123	133	0.96 (0.74 - 1.24)	64	65	1.01 (0.70 - 1.46)
≥150 mg/day	52	40	0.83 (0.55 - 1.26)	24	18	0.76 (0.42 - 1.38)
Recent DPP4-I use	131	168	0.76 (0.59 - 0.97)*	63	90	0.71 (0.50 - 1.01)
Past DPP4-I use	181	186	0.96 (0.77 - 1.20)	96	106	0.96 (0.71 - 1.30)
Distant past DPP4-I use	112	121	0.95 (0.72 - 1.25)	59	66	0.90 (0.61 - 1.34)

CI: confidence interval; DPP4-I: dipeptidyl peptidase 4 inhibitor; GLP1-RAs: glucagon-like peptide 1 receptor agonists: NIAD: non-insulin anti-diabetic-drugs; OR: odds ratio. Current NIAD use: Most recent NIAD prescription within 91 days before index date. Current DPP4-I use: Most recent DPP4-I prescription within 91 days before index date. Recent DPP4-I use: Most recent DPP4-I prescription within 92 - 182 days before index date. Past DPP4-I use: Most recent DPP4-I prescription within 183 - 365 days before index date. Distant DPP4-I use: Most recent DPP4-I prescription more than 365 days before index date.* Statistically significant, (P<0.05). [a] The numbers do not sum to the total number of fractures since never incretin use, past NIAD use and GLP1-RA exposure are not shown. [b] Adjusted for use history of cancer, alcoholism, chronic obstructive pulmonary disease, fracture, rheumatoid arthritis, hyperthyroidism, secondary osteoporosis, retinopathy, neuropathy and heart failure and use of GLP1-RAs, glucocorticoids, statins, antidepressants, anxiolytics, hypnotics, antipsychotics, anti-Parkinson drugs, beta-blockers, thiazide diuretics, renin angiotensin aldosterone system inhibitors, loop diuretics and antiarrhythmics. [c] Adjusted for (b), but not for hyperthyroidism and neuropathy. [d] Expressed as sitagliptin equivalents.

We note that our results are in contrast to a meta-analysis on the effect of DPP4-Is on fracture[12] identifying that DPP4-I use was associated with a 40% reduction in fracture risk. However, this meta-analysis had several limitations, which may explain the discrepancies with our findings. Specifically, the mean duration of follow-up was only 35 weeks, the number of fractures was low (n=63) and the outcome was not routinely collected. Based on the low number of fractures and the short duration an attenuation of the effect would be expected, nevertheless a 40% reduced fracture risk was found. In addition, 12 weeks of sitagliptin treatment was associated with a decreased bone resorption marker as compared to metformin use in postmenopausal diabetic women, which may support a beneficial effect of DPP4-Is[29].

Different mechanisms by which GLP-1 might result in increased bone formation[30-32] have been previously hypothesized. GLP-1 and glucose-dependent insulinotropic polypeptides (GIP) are incretin hormones that are secreted by the intestine after food ingestion, whereas DPP4 is an enzyme that degrades incretin hormones quickly after secretion[3]. DPP4-Is inhibit the enzymal degradation process and thereby prolongs the half-life time of the incretin hormones.

In vitro studies have identified GLP-1 receptors on bone marrow stromal cells[33], immature osteoblast[10] and osteocytes[9]. Activation of the GLP-I receptor has shown to lead to increased osteoblast activity[30] and inhibition of bone resorption[34] that, theoretically, may lead to elevated bone formation and a reduced fracture risk. Similarly, GIP receptors have been identified on osteoblasts[11], osteoclasts[35], and bone marrow stromal cells[36]. Binding of GIP to its receptor stimulates osteoblast activity and inhibits osteoclast activity[10,11], which may increase bone formation and reduce fracture risk. Decreased bone turnover has been associated with increased microdamage accumulation in animal studies[37]. This might result in an increased risk of fracture because the bones become more brittle. So, it might be hypothesized that use of DPP4-Is may result in an increased risk of fracture due to the decreased bone turnover. However, a study investigating the frequency of cancellous bone microcracks in patients who had used bisphosphonates for more than three years as compared to controls did not show a different frequency of microcracks[38]. In summary, more research is needed to better elucidate whether DPP4-Is are able to increase bone formation via the prolonging of the half-life of GLP-1 and GIP, and whether this effect is sufficient to alter osteoporotic fracture risk in humans.

Recent DPP4-I use showed a decreased association with risk of any fracture and risk of vertebral fracture. Surprisingly, this was not found for other DPP4-I users (current, past and distant), which would have been expected when an underlying mechanism is causing this decreased fracture risk. Consequently, we consider these results to likely be due to chance, and believe they require further replication. The total number of vertebral fractures with recent DPP4-I use was rather low and these results should therefore be interpreted with caution. Never and past NIAD use had an increased fracture risk as compared to current use of other anti-hyperglycaemic drugs, which was

also unexpected. Unfortunately, we were unable to adjust our analysis for body mass index (BMI), which has been shown to decrease fracture risk[39]. Since an elevated BMI is a risk factor for diabetes, our finding that the increased risk associated with never NIAD use is a by-product of higher BMI among the current NIAD users. We also note that our past NIAD user group includes patients with type 2 diabetes mellitus receiving insulin therapy. Prior evidence has identified that fracture risk doubles for type 2 diabetes mellitus patients who use insulin compared to patients who receive strictly oral mono-therapy[2].

Strengths of this study include the nation-wide population-based design, large sample size, the validity of data, and the completeness of the Danish Health Registries. In addition, fractures are well captured in the hospital system of Denmark[15]. Another strength is the fact that data are prospectively collected, which circumvents any recall bias. Unfortunately, there was no data available about life style factors, such as BMI and smoking, or lab tests. For example, glycosylated hemoglobin A1c (HbA1c) values, which might be potential confounders, were not captured in our data. However, as a proxy for HbA1c/poor diabetes control, we corrected our analyses for known complications of diabetes, such as neuropathy and retinopathy, and we used current use of other anti-hyperglycaemic drugs (with the exception of insulin) as a proxy for diabetes. However, it is acknowledged that some residual confounding may still be present. For example, current NIAD use included current TZD and insulin use, which has been associated with an elevated fracture risk[4-7]. The result of this bias in the reference category may be an observed artificial inverse association between DPP4-I use and risk of fracture, which could have falsely supported our hypothesis. However, we did not observe an inverse association between DPP4-I use and risk of fracture. We might not have been able to fully adjust for diabetes severity which could have masked a true decreased risk of fracture with use of DPP4-Is. When studying a population with diabetes patient only, the adjusted diabetic complication severity index may be used to correct for diabetes severity[40]. We further note that metformin use has been associated with a reduced fracture risk. While our referent group included current metformin users, which may have limited our findings, we did not find a decreased risk of fracture associated with current use of DPP4-Is after adjusting for current metformin use. Another limitation of our study was the relatively short mean duration (47 weeks) of DPP4-I use (time from first to index date). However, a meta-analysis of clinical trials investigating the effect of DPP4-I use on risk of fracture showed a decreased effect on fracture risk with a mean duration of 35 weeks[12].

In summary, we showed in a population-based case-control study that short-term DPP4-I use was not associated with fracture risk as compared to use of other anti-hyperglycaemic drugs. Additionally, increasing daily dose and cumulative DPP4-I exposure were also not associated with fracture risk. However, more research is needed to assess the effect of long-term DPP4-I use on the risk of fracture.

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2.3

Chapter 2.4

Bone fracture risk is not associated with the use of glucagon-like peptide 1 receptor agonists: a population-based cohort analysis

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Abstract

Introduction

Glucagon-like peptide 1 receptor agonists (GLP1-RAs) are a relatively new class of anti-hyperglycaemic drugs which may positively affect bone metabolism and thereby decrease (osteoporotic) bone fracture risk. Data on the effect of GLP1-RAs on fracture risk are scarce and limited to clinical trial data only. The aim of this study was to investigate, in a population-based cohort, the association between the use of GLP1-RAs and bone fracture risk.

Methods

We conducted a population-based cohort study, with the use of data from the Clinical Practice Research Datalink (CPRD) database (2007-2012). The study population (n=216,816) consisted of all individuals with type 2 diabetes mellitus patients with at least one prescription for a non-insulin anti-diabetic drug (NIAD) and were over 18 years of age. Cox proportional hazards models were used to estimate the hazard ratio of fracture in GLP1-RA users versus never-GLP1-RA users. Time-dependent adjustments were made for age, sex, lifestyle, comorbidity and the use of other drugs.

Results

There was no decreased risk of fracture with current use of GLP1-RAs as compared to never-GLP1-RA use (adjusted hazard ratio (HR) 0.99, 95% CI: 0.82-1.19). Osteoporotic fracture risk was also not decreased by current GLP1-RA use (adjusted HR 0.97, 95% CI: 0.72-1.32). In addition, stratification according to cumulative dose did not show a decreased bone fracture risk with increasing cumulative GLP1-RA dose.

Conclusion

We showed in a population-based cohort study that GLP1-RA use is not associated with a decreased bone fracture risk as compared to users of other anti-hyperglycaemic drugs. Future research is needed to elucidate the potential working mechanisms of GLP1-RAs on bone.

Introduction

In individuals with type 2 diabetes mellitus (T2DM), the risk of bone fracture is increased as compared to individuals without T2DM[1]. This increased risk might be associated with the pathobiology of T2DM itself, although the underlying mechanisms remain largely unknown[2]. Alternatively, it has been suggested that this increased bone fracture risk is a consequence of the kind of therapeutic regimen initiated to combat hyperglycaemia[3]. For instance, thiazolidinediones[4-6] have shown to increase fracture risk. It has been suggested that human recombinant insulins[7] also increase bone fracture risk, whereas metformin might actually decrease it[8].

Glucagon-like peptide 1 receptor agonists (GLP1-RAs) are a relative new class of anti-hyperglycaemic drugs, which may positively affect bone metabolism[9-11] and decrease bone fracture risk. However, a recent meta-analysis, based upon randomised clinical trial data, in which bone fractures were not a primary outcome, did not show a decreased bone fracture risk[12]. Thus, the aim of the present study was to investigate, in a large population-based cohort study, the association between GLP1-RA use and the risk of bone fractures in individuals with T2DM.

Methods

Data source

Data were obtained from the British Clinical Practice Research Datalink (CPRD; previously the General Practice Research Database [GPRD]; see [www.CPRD.com]). The CPRD contains computerized medical records of 625 primary care practices in the United Kingdom (UK), and patients represent 8% of the UK population. Data have been collected since 1987 and include, amongst others; demographic information, prescription details, data on morbidity and mortality, preventive care provided and specialist referrals. Data in the CPRD has been shown to be accurate and valid[13]. Particularly, with regard to the main outcome of the present study, fractures have been validated in over 90% of all cases[14].

Study population

We conducted a population-based cohort study. The population consisted of all patients with at least one prescription for a non-insulin anti-hyperglycaemic drug (NIAD) and who were aged 18+ during the period of valid CPRD data collection[15]. Cohort entry was defined as the date of first prescription for GLP1-RA, identified between June 13th, 2007 and August 31, 2012. The index date was defined as the date of the first NIAD prescription; thereby the study population was a mix of incident and prevalent NIAD users. Patients were followed from the index date to the end of data

collection, date of transfer of the patient out of the practice area, patient's death, or fracture types of interest, whichever came first.

Exposure

Follow-up time was divided into intervals based on NIAD and insulin prescriptions. Thus a new interval was created for every prescription. When there was a washout period of 90 days, an interval was classified as “past use”, until end of follow-up, or a new anti-hyperglycaemic drug prescription, whichever came first. In all other circumstances an interval was classified as “current use”. If no GLP1-RA was prescribed during follow-up person-time was classified as never use of GLP1-RA.

All GLP1-RA exposed intervals were classified, according to the time since the most recent prescription, as current (1-90 days), recent (91-180 days), or past (over 180 days) GLP1-RA use. At every current use interval, the cumulative prescribed GLP1-RA dosage, in exenatide dose equivalents, was reviewed and divided by the GLP1-RA treatment time (difference in time between the start of the first and last GLP1-RA prescription) to estimate the average daily GLP1-RA dose. Defined daily doses were used to calculate the exenatide dose equivalents[16].

Outcomes

Patients were followed from the index date to the end of data collection, date of transfer of the patient out of the practice area, patient's death, or fracture types of interest, whichever came first. Fractures were classified with the use of READ codes[17] into hip, radius and (or) ulna, vertebral, humerus and other fractures. A major osteoporotic fracture was defined according to the WHO definition as a fracture of the hip, humerus, vertebral or radius/ulna[18].

Other variables

The presence of risk factors for bone fractures was assessed by reviewing the computerized medical records for any record of any risk factors for bone fractures prior to the start of an interval. The following potential confounders were determined at baseline: sex, body mass index (BMI), smoking status, and alcohol use. All other potential confounders that were considered in this study were determined time-dependent (i.e. at the start of each interval): age, falls in 7 to 12 months before the start of an interval, a history of chronic obstructive pulmonary disease, a previous fracture, rheumatoid arthritis, hypothyroidism, hyperthyroidism, cancer, retinopathy, neuropathy, secondary osteoporosis (hypogonadism or early menopause), and congestive heart failure. The most recent glycosylated hemoglobin type A1c (HbA1c) value up to one year prior to the start of an interval was determined. The following drug prescriptions, in the 6 months prior to the start of an interval, were considered

potential confounders; oral glucocorticoids[19], cholesterol lowering drugs, antidepressants[20], anxiolytics or hypnotics[21], antipsychotics, anti-Parkinson drugs[22], antihypertensives (beta-blockers, thiazide diuretics, renin angiotensin aldosterone system inhibitors, calcium channel blockers, loop diuretics), antiarrhythmics, hormone replacement therapy, calcium, bisphosphonates, vitamin D, raloxifene, strontium ranelate, calcitonin, and parathyroid hormone.

Statistical analyses

Regression analysis with Cox proportional hazards models (SAS 9.2, PHREG procedure) was used to estimate bone fracture risk of GLP1-RA users (current, recent or past) compared to never-GLP1-RA users. Current GLP1-RA use was further stratified to age and gender and the main analyses was repeated for major osteoporotic bone fracture risk. In a series of further analyses, current GLP1-RA use was stratified according to type of GLP1-RA (i.e. exenatide and liraglutide), daily and cumulative dose. As a sensitivity analysis the person-time for thiazolidinediones (TZD) was excluded from the reference group and analysed as a separate group.

In all analyses potential confounders were included if they independently changed the beta-coefficient for current GLP1-RA exposure by at least 5%, or when consensus about inclusion existed within the team of researchers, supported by clinical evidence from literature.

Results

Study population and follow-up

In total, 216,816 individuals were included in the present study, of which 8,354 used GLP1-RAs (either current, recent or past). The characteristics of the study population are presented in Table 2.4.1. On average, GLP1-RA users were younger than never-GLP1-RA users (53.5 vs. 61.0 years), and had a higher BMI (37.5 vs. 31.0). The median duration of follow-up time (from start of follow-up to end of data collection) was 5.1 years (Inter Quartile Range [IQR]: 3.6-5.2 years for GLP1-RA users and 3.6 years (IQR: 1.6-5.2 years) for never-GLP1-RA users.

After adjusting for confounders, the risk for bone fractures with current GLP1-RA, as compared to never-GLP1-RA users, was (hazard ratio [HR] and [95% CI]): 0.99 (95% CI: 0.82-1.19), with recent GLP1-RA use: 1.19 (95% CI: 0.66-2.14) and with past GLP1-RA use: 1.38 (95% CI: 1.03-1.84), Table 2.4.2. Stratification of current GLP1-RA by GLP1-RA type resulted in an adjusted (adj.) HR of 0.90 (95% CI: 0.69-1.17) with use of liraglitude and an adj. HR of 1.08 (95% CI: 0.84-1.38) with exenatide use.

Table 2.4.1 Baseline characteristics of GLP1-RA users and never-GLP1-RA users[a].

Characteristic	GLP1-RA users[b] N=8,354	Never-GLP1-RA users[c] N=208,462
Median [IQR] follow-up time, years	5.1 [3.6 - 5.2]	3.6 [1.6 - 5.2]
Median [IQR] duration of actual GLP1-RA use, years	1.7 [0.8 - 2.7]	n/a
Women	3,904 (46.7)	98,614 (47.3)
Age		
Mean age at index date (years, SD)	53.5 (10.5)	61.0 (15.1)
18 - 49 years	2,952 (35.3)	43,574 (20.9)
50 - 59 years	2,909 (34.8)	41,717 (20.0)
60 - 69 years	1,986 (23.8)	54,865 (26.3)
70 - 79 years	481 (5.8)	46,907 (22.5)
80+ years	26 (0.3)	21,399 (10.3)
BMI		
Mean BMI at index date (kg/m^2, SD)	37.5 (7.1)	31.0 (6.5)
<20.0 kg/m^2	3 (0.0)	2,759 (1.3)
20.0 - 24.9 kg/m^2	84 (1.0)	26,969 (12.9)
25.0 - 29.9 kg/m^2	936 (11.2)	67,550 (32.4)
30.0 - 34.9 kg/m^2	2,369 (28.4)	57,826 (27.7)
35.0 kg/m^2	4,921 (58.9)	48,016 (23.0)
Missing	41 (0.5)	5,342 (2.6)
HbA1c %		
Mean (SD)	8.6 (1.9)	8.0 (1.8)
Missing	2,915 (34.9)	88,937 (42.7)
Smoking status		
Never	3,949 (47.3)	103,970 (49.9)
Current	1,945 (23.3)	41,110 (19.7)
Ex	2,458 (29.4)	62,287 (29.9)
Missing	2 (0.0)	1,095 (0.5)
Alcohol use		
No	2,417 (28.9)	60,278 (28.9)
Yes	5,638 (67.5)	136,373 (65.4)
Missing	299 (3.6)	11,811 (5.7)
Falls (7 - 12 months before)	52 (0.6)	2,141 (1.0)
History of diseases		
Fracture	52 (0.6)	1,342 (0.6)
Hyperthyroidism	64 (0.8)	2,014 (1.0)
Hypothyroidism	719 (8.6)	16,413 (7.9)
COPD	345 (4.1)	11,585 (5.6)
Congestive heart failure	244 (2.9)	8,846 (4.2)
Cancer	1,626 (19.5)	45,626 (21.9)
Rheumatoid arthritis	109 (1.3)	3,633 (1.7)
Retinopathy	1,284 (15.4)	24,954 (12.0)
Secondary osteoporosis	895 (10.7)	18,571 (8.9)
Neuropathy	768 (9.2)	15,652 (7.5)
Drug use within six months before index date		
Metformin	7,367 (88.2)	171,699 (82.4)
Sulfonylurea derivatives	4,589 (54.9)	54,874 (26.3)
Thiazolidinediones	2,108 (25.2)	20,293 (9.7)
Insulin	2,238 (26.8)	22,556 (10.8)
DPP4-Is	144 (1.7)	1,374 (0.7)
Glucocorticoids	439 (5.3)	12,077 (5.8)

Table 2.4.1 (continued)

Characteristic	GLP1-RA users[b] N=8,354	Never-GLP1-RA users[c] N=208,462
Statins	5,803 (69.5)	113,343 (54.4)
Antiarrhythmics	125 (1.5)	3,336 (1.6)
Antidepressants	2,075 (24.8)	32,995 (15.8)
Anti-Parkinson drugs	24 (0.3)	1,040 (0.5)
Antipsychotics	157 (1.9)	4,343 (2.1)
Anxiolytics	658 (7.9)	14,752 (7.1)
Hypnotics	437 (5.2)	10,242 (4.9)
Antihypertensives	5,651 (67.6)	120,188 (57.7)
Bisphosphonates	87 (1.0)	5,569 (2.7)
Raloxifene	5 (0.1)	298 (0.1)
Vitamin D	42 (0.5)	1,161 (0.6)
Calcium	145 (1.7)	8,263 (3.9)
Strontium	0 (-)	124 (0.1)
PTH/calcitonin	0 (-)	0 (-)
Hormone replacement therapy	71 (0.8)	835 (0.4)

BMI: body mass index; COPD: chronic obstructive pulmonary disease; DPP4-I: dipeptidyl peptidase 4 inhibitor; GLP1-RA: glucagon-like peptide 1 receptor agonist; IQR: inter quartile range; NIAD: non-insulin anti-diabetic drug, PTH: parathyroid hormone; SD: standard deviation. [a] Data are number (%) of patients, unless stated otherwise. [b] GLP1-RA users are patients who had at least 1 GLP1-RA prescription during follow-up. [c] Never-GLP1-RA users are patients who had at least 1 NIAD prescription other than GLP1-RA, during follow-up.

When stratified by sex, bone fracture risk for current male GLP1-RA users was 1.01 (95% CI: 0.76-1.33) and for current female GLP1-RA users was 0.96 (95% CI: 0.75-1.22), Table 2.4.2. After stratification by age group (i.e., 18-49, 50-59, 60-69 and ≥70 years), the risk for bone fractures among patients with current GLP1-RA use aged 18-49 was 0.80 (95% CI: 0.52-1.25), aged 50-59 was 0.80 (95% CI: 0.56-1.15), aged 60-69 was 1.38 (95% CI: 1.04-1.83), and aged 70 years or older was 0.68 (95% CI: 0.39-1.18), Table 2.4.2.

As compared to never-GLP1-RA use, the risk for major osteoporotic fractures with current GLP1-RA use was 0.97 (95% CI: 0.72-1.32), with recent GLP1-RA use; 1.13 (95% CI: 0.42-3.02) and with past GLP1-RA use; 1.04 (95% CI: 0.61-1.76), Table 2.4.3 (detailed data not shown). Current GLP1-RA use was not associated with a decreased bone fracture risk for other fracture types, Table 2.4.3.

If current GLP1-RA use was stratified according to cumulative dose (i.e., 0-2.7 mg, 2.8-5.4 mg, 5.5-8.2 mg and ≥8.3 mg exenatide dose equivalents) the results showed that, as compared to never-GLP1-RA users, bone fracture risk for 0-2.7 mg was 1.02 (95% CI: 0.75-1.40), for 2.7-5.5 mg; 0.89 (95% CI: 0.60-1.34), for 5.5-8.2 mg; 0.94 (95% CI: 0.58-1.52) and for ≥8.3 mg; 1.03 (95% CI: 0.75-1.41). When stratified according to current GLP1-RA average daily dose use (i.e., missing, 0-15 mcg, 16-20 mcg and >20 mcg exenatide dose equivalents) the results were not substantially altered, Table 2.4.4.

Table 2.4.2 Risk of bone fracture in GLP1-RA users compared with never-GLP1-RA users[a].

	No. of fractures N=9,340[b]	Fracture IR (/1000 PY)	Age/sex adjusted HR (95% CI)	Adjusted HR[d] (95% CI)
GLP1-RA exposure				
Never use[c]	8,449	12.9	Reference	Reference
Past use	47	16.1	1.48 (1.11 - 1.95)*	1.38 (1.03 - 1.84)*
Recent use	11	13.4	1.22 (0.69 - 2.14)	1.19 (0.66 - 2.14)
Current use	122	10.6	1.00 (0.83 - 1.20)	0.99 (0.82 - 1.19)
By GLP1-RA type				
Liraglutide	57	9.5	0.91 (0.70 - 1.18)	0.90 (0.69 - 1.17)
Exenatide	65	11.8	1.09 (0.85 - 1.40)	1.08 (0.84 - 1.38)
By sex[e]				
Men	53	8.5	1.02 (0.78 - 1.35)	1.01 (0.76 - 1.33)
Women	69	13.1	0.98 (0.77 - 1.25)	0.96 (0.75 - 1.22)
By age[f]				
18 - 49	22	7.6	0.88 (0.57 - 1.35)	0.80 (0.52 - 1.25)
50 - 59	33	8.5	0.83 (0.58 - 1.19)	0.80 (0.56 - 1.15)
60 - 69	54	15.0	1.48 (1.12 - 1.95)*	1.38 (1.04 - 1.83)*
70+	13	11.3	0.60 (0.35 - 1.04)	0.68 (0.39 - 1.18)

CI: confidence interval; DPP4-I: dipeptidyl peptidase 4 inhibitor; GLP1-RA: glucagon-like peptide 1 receptor agonist; HR: hazard ratio; IR: incidence rate; NIAD: non-insulin anti-diabetic drug. Current GLP1-ra use: most recent prescription within 90 before start of an interval. Recent GLP1-ra use: most recent prescription within 91–180 before start of an interval. Past GLP1-ra use: most recent prescription over 180 days before start of an interval. * Statistically significant, (P<0.05). [a] All models are corrected for DPP4-I use. [b] Past NIAD use not shown, therefore the total number of fractures do not add up. [c] Never GLP1-RA use does not include use of DPP4-I. [d] Adjusted for sex, age, body mass index, smoking status, glycosylated hemoglobin type A1c, retinopathy, neuropathy, secondary osteoporosis, and the use of glucocorticoids, cholesterol lowering drugs, hypnotic/anxiolytic drugs and antidepressants. [e] Models not statistically adjusted for sex. [f] Models not statistically adjusted for age.

As TZD use has been associated with an increased risk of fracture we additionally excluded for the main analysis all TZD exposed person-time from the reference group and analysed it as a separate group. This did not substantially change the results of GLP1-RA use (adj. HR with current GLP1-RA use; 1.03 (95% CI: 0.86-1.24), with recent GLP1-RA use; 1.19 (95% CI: 0.66-2.15) and with past GLP1-RA use; 1.38 (95% CI: 1.03-1.84)).

Table 2.4.3 Risk of bone fracture in current GLP1-RA users stratified to fracture type[a].

Fracture sites	No. of fractures	Fracture IR (/1000 PY)	Age/sex adjusted HR (95% CI)	Adjusted HR (95% CI)
Major osteoporotic fracture				
Never-GLP1-RA use[b]	4,373	6.1	Reference	Reference
Current GLP1-RA use	44	3.7	0.97 (0.71 - 1.31)	0.97 (0.72 - 1.32)[c]
Hip fracture				
Never-GLP1-RA use[b]	1,383	1.9	Reference	Reference
Current GLP1-RA use	2	0.2	0.26 (0.06 - 1.03)	0.27 (0.07 - 1.08)[d]
Radius/ulna fracture				
Never-GLP1-RA use[b]	1,442	2.0	Reference	Reference
Current GLP1-RA use	15	1.3	1.78 (0.47 - 1.32)	0.82 (0.48 - 1.37)[e]
Vertebral fracture				
Never-GLP1-RA use[b]	513	0.7	Reference	Reference
Current GLP1-RA use	8	0.7	1.59 (0.78 - 3.24)	1.64 (0.80 - 3.37)[f]

CI, confidence interval; DPP4-I, dipeptidyl peptidase 4 inhibitor; GLP1-RA, glucagon-like peptide 1 receptor agonist; HR: hazard ratio; IR: incidence rate. Current GLP1-ra use: most recent prescription within 90 before start of an interval. * Statistically significant, (P<0.05). [a] All models are corrected for DPP4-I use. [b] Never-GLP1-RA use does not include use of DPP4-I. [c] Adjusted for (g), and history of congestive heart failure, use of cholesterol lowering drugs, antidepressants, hypnotics/anxiolytics, calcium, and anti-osteoporotic drugs[g]. [d] Adjusted for (g), and the use of glucocorticoids, antidepressants, and falls 7-12 months before index date. [e] Adjusted for (g), and the use of cholesterol lowering drugs, antidepressants, hypnotics/anxiolytics, anti-osteoporotic drugs[h], and glucocorticoids. [f] Adjusted for (g), and history of congestive heart failure, use of cholesterol lowering drugs, antidepressants, hypnotics/anxiolytics, and glucocorticoids. [g] Sex, age, body mass index, glycosylated hemoglobin type A1c, smoking status, HbA1c, retinopathy, neuropathy and secondary osteoporosis. [h] Use of bisphosphonates, raloxifene, strontium ranelate or parathyroid hormone/calcitonin.

Table 2.4.4 Risk of bone fracture in current GLP1-RA users compared to never-GLP1-RA users, stratified by average and cumulative DDD exposure[a].

	No. of fractures	Fracture IR (/1000 PY)	Age/sex adjusted HR (95% CI)	Adjusted HR (95% CI)[c]
Never-GLP1-RA use[b]	8,449	12.9	Reference	Reference
Current GLP1-RA use				
By average DDD exposure (in exenatide equivalents)				
0 - 15 mcg	48	16.9	1.05 (0.79 - 1.40)	1.03 (0.78 - 1.38)
16 - 20 mcg	37	8.7	0.82 (0.59 - 1.14)	0.82 (0.59 - 1.13)
≥21 mcg	28	14.3	1.29 (0.89 - 1.88)	1.27 (0.88 - 1.85)
No dose	9	9.8	0.91 (0.48 - 1.76)	0.90 (0.47 - 1.73)
By cumulative DDD exposure (in exenatide equivalents)				
0 - 2.7 mg	41	11.3	1.03 (0.76 - 1.41)	1.02 (0.75 - 1.40)
2.8 - 5.4 mg	24	9.4	0.90 (0.60 - 1.35)	0.89 (0.60 - 1.34)
5.5 - 8.2 mg	17	9.9	0.95 (0.59 - 1.53)	0.94 (0.58 - 1.52)

CI: confidence interval; DDD: defined daily dose; DPP4-I: dipeptidyl peptidase 4 inhibitor, GLP1-RA: glucagon-like peptide 1 receptor agonist; HR: hazard ratio; IR: incidence rate, NIAD: non-insulin anti-diabetic drug. [a] Also corrected for DPP4-I use. [b] NIAD past use, GLP1-RA recent and GLP1-RA past use not shown. Never-GLP1-RA use does not include use of DPP4-I. [c] Adjusted for sex, age, body mass index, smoking status, glycosylated hemoglobin type A1c, history of secondary osteoporosis, retinopathy and neuropathy, use of glucocorticoids, cholesterol lowering drugs, hypnotic/anxiolytic drugs and antidepressants.

Discussion

The results of the present population-based study show that (osteoporotic) bone fracture risk was not decreased by GLP1-RA use. In addition, stratification according to cumulative dose did not show a decreased risk of bone fracture with increasing cumulative GLP1-RA dose. The results of the present study thereby do not support the hypothesis that GLP1-RA use may reduce bone fracture risk in individuals with T2DM. The results of our study add to the field population-based data and are indirectly supported by a clinical trial on the effect of exenatide on markers of bone remodelling and calcium homeostasis, which failed to show a positive effect[23]. Our study is thereby in line with a recent meta-analysis[12] done on randomised clinical trial data. Another more recent meta-analysis also showed no association between use of GLP1-RAS and fracture risk[24]. However, after stratification to GLP1-RA type they found a decreased risk of fracture with use of liraglutide and an increased risk of fracture with use of exenatide. The results of our study did not show a decreased or increased risk after stratification by GLP1-RA type. It has to be taken into account, however, that the included studies of these meta-analyses were not designed to investigate fracture risk and that fractures were not routinely registered. The results of our study are also in keeping with the results of a large cohort study on the use of dipeptidyl peptidase 4 inhibitors (DPP4-Is) and fracture risk which also did not show a decreased fracture risk[25].

The pathways through which GLP1-RAs may act on bone metabolism are not fully elucidated, but it has been suggested that GLP1-RAs may, either directly or indirectly, shift the balance in bone homeostasis towards bone formation[26], via receptor coupling on osteoblasts[27] and (or) thyroid C cells[28,29]. Alternatively, it has been suggested that GLP1-RA may increase calcitonin concentration[29,30] and decreases sclerostin which both may inhibit bone formation[31]. Nevertheless, it remains to be determined whether such mechanisms may also be operative in humans. Interestingly, a recent meta-analysis of clinical trial data on the use of DPP4-Is did show a 40% reduction in the risk of bone fracture[32]. The latter brings forward the hypothesis that any effects on bone metabolism by DPP4-I might be independent from the direct effect of GLP1 on bone, despite the pharmacodynamics through which they are linked[3]. However, the underlying mechanisms between anti-hyperglycaemic drug use along the GLP1/DPP4 axis and bone fracture risk in T2DM in humans remain complex.

Unexpectedly, our results showed a 1.4-fold increase in bone fracture risk for past GLP1-RA users (GLP1-RA use had discontinued ≥180 days) and for patients aged 60-69 compared to never-GLP1-RA users. As any plausible underlying mechanism seems missing, we consider these results as a play of chance.

Our study had several strengths. Firstly, the results were based upon population-based data that may have prevented, at least partially, selection bias as compared to randomised clinical trials in which only patients meeting specific inclusion criteria are

able to participate. Secondly, bone fractures are often not primary end-points in clinical trials, thus their registration may be inadequate. The current study, however, was able to partially circumvent this potential bias, as over 90% of all fractures have been clinically validated in the CPRD[14]. Finally, all participants were extensively clinically characterized which allowed us to take a series of potential confounders, including prior medical history, into account. In addition we were able to adjust for neuropathy, retinopathy and HbA1c with which we tried to capture disease severity. In particular, HbA1c could act as a potential confounder of the association between GLP1-RA use and bone fracture risk in individuals with T2DM. However, it is acknowledged that some residual confounding may still be present.

When interpreting the results, a couple of limitations are worth mentioning. First, an important consideration, in light of the discussion on GLP1-RA use and bone fracture risk, in terms of time effect, is the average duration of GLP1-RA use. In our analysis the median duration of actual GLP1-RA use was 1.2 years (from first GLP1-RA prescription until last GLP1-RA prescription), and this might be relatively short. For bisphosphonate use it has been shown that bone fracture risk starts to decrease after 1 to 1.5 years of use[33,34]. Even for the highest cumulative dose group (i.e., ≥8.3 mg), which could be equivalent to 1 DDD GLP1-RA per day during at least 1.5 years, we did not show a decrease in bone fracture risk. However, the time-window for GLP1-RA to exert an effect on bone fracture risk is not yet determined. Second, the risk of fracture is known to increase with age[35], yet we identified that patients who used GLP1-RA were slightly younger at baseline, as compared to never-GLP1-RA users. Thus, this might have masked the protective effect of GLP1-RA on risk of bone fracture. Yet, our age-stratified analyses did not show a protective effect of GLP1-RA use on bone fracture risk. Third, GLP1-RAs are selectively prescribed to patients with a high BMI, which might have influenced the results. High BMI has been associated with a lower risk of fracture[36], and this might even strengthen the protective effect of GLP1-RAs. However, we could not show a protective effect of GLP1-RAs on fracture risk. Fourth, after stratification of the analyses to specific fracture types the number of fractures within the current GLP1-RA group became low and therefore the results should be interpreted with caution.

In summary, we showed in a population-based cohort study that GLP1-RA use is not associated with a decreased risk of bone fracture as to users of other anti-hyperglycaemic drugs. Future research is needed to elucidate the working mechanisms of the complex GLP1/DPP4 axis and to investigate the time-window of GLP1-RA to exert an effect on bone fracture risk.

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2.4

Chapter 2.5

Use of glucagon-like peptide 1 receptor agonists and risk of fracture as compared to use of other anti-hyperglycaemic drugs

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Abstract

Introduction

Glucagon-like peptide 1 receptor agonists (GLP1-RAs) are a new class of drugs that might have a potential beneficial effect on bone metabolism. Data on the effect of GLP1-RAs and fracture risk is lacking. The aim of the present study was to investigate the association between the use of GLP1-RAs and the risk of fracture.

Methods

A case-control study was performed using Danish National Health Service data. Cases were those who sustained a fracture and controls were those without a fracture during the study period (2007-2011), all aged 18 years and older. Conditional logistic regression estimated the odds ratios of fracture with current use of GLP1-RAs. Analyses were adjusted for comorbidities and recent drug use.

Results

Among cases (n=229,114), there were 6,993 current non-insulin anti-diabetic drug (NIAD) users (excluding incretin users) and 255 GLP1-RA users. Similarly, among controls (n=229,114), 7,209 were NIAD users (excluding incretin users) and 220 were GLP1-RA users. Current GLP1-RA use was not associated with a decreased risk of fracture (adjusted (adj.) odds ratio (OR): 1.16 (95% CI: 0.83-1.63)). Osteoporotic fracture risk was also not associated with current GLP1-RA use (adj. OR: 0.78 (95% CI: 0.44-1.39)).

Conclusion

In our nation-wide case-control study we identified that the use of GLP1-RA was not associated with fracture risk as compared to use of other anti-hyperglycaemic drugs. Additionally, current GLP1-RA use, stratified by cumulative or average daily dose, is not associated with fracture risk. Further research should focus on long-term use of GLP1-RAs and fracture risk.

Introduction

The risk of fracture is significantly increased in patients with type 2 diabetes mellitus (T2DM)[1]. It has been hypothesized that a reduced bone strength or bone quality plays a role in patients with T2DM[2]. Alternatively, it has been suggested that anti-hyperglycaemic drugs might affect fracture risk. For instance, observational studies showed that use of thiazolidinediones is associated with a 1.3-1.9 fold[3,4] increased risk of fracture, in particular in women, as compared to use of other anti-hyperglycaemic drugs. Insulin use has been associated with an elevated fracture risk[5] whereas metformin might be associated with a reduced fracture risk[6].

Glucagon-like peptide 1 receptor agonists (GLP1-RAs), such as exenatide and liraglutide, are a new class of drugs in the treatment of T2DM. GLP1-RAs may have a potential beneficial effect on bone metabolism, established by binding of GLP1-RAs to a glucagon-like peptide 1 (GLP1) receptor on osteocytes and osteoblasts, as shown in in vitro studies[7-9]. Consequently, this may result in an increased bone formation and a decreased bone resorption[10,11]. As a result of this process, we hypothesize that this then may lead to a reduced risk of fracture.

However, current there is limited data, particularly epidemiologic, in the literature. A recent meta-analysis of randomised clinical trials (n=4,255, mean duration of included studies 67.4 weeks) did not show a reduced risk of fracture with use of GLP1-RAs[12] as compared to use of other anti-hyperglycaemic drugs. To our knowledge there is only one observational study examining fracture risk with GLP1-RA use which showed no association[13]. In particular, studies with longer durations of use or stratified to daily dose are required to best understand the association between GLP1-RA use and fracture risk. We therefore sought to examine the association between GLP1-RA use compared to other anti-hyperglycaemic drug use and fracture risk in a nation-wide case-control study.

Methods

Data source

We utilized data from the Danish National Health Service, which covers all contacts to the health sector, and includes approximately 5.2 million individuals in 1995 and 5.5 million in 2011[14]. The unique 10-digit civil registry number was used to link population-based registries and generate a complete hospital discharge and prescription history for each individual[15]. Data on vital status (e.g., change of address and date of death) for the entire Danish population have been collected since 1968 in the Civil Registration System. All inpatient contacts have been registered through the Danish National Hospital Discharge Register[16] since 1977, and outpatient visits to hospitals, outpatient clinics, and emergency rooms have been included since 1995. In

Denmark, prescription records are sent directly to a Register of Medicinal Product Statistics (i.e. a prescription database) at the Danish Medicines Agency. The prescription database includes information on patient's civil registry number, the type and amount of drug prescribed according to the Anatomical Therapeutic Chemical (ATC) classification system and the date when the prescription was filled. Pharmacy data have been collected since January 1, 1996. This study was subject to control by the National Board of Health and the Danish Data Protection Agency.

Study design

The study was designed as a case-control study. Cases were all subjects, both genders and aged 18 years and older, who sustained a fracture, low or high trauma, (International Classification of Diseases and Related Health Problems (ICD)-10 codes: S02, S12, S22, S32, S42, S52, S62, S72, S82, S92, T02, T08, T10, and T12) between 9 May 2007 (the first ever prescription of a GLP1-RA in Denmark) and 31 December 2011. Controls were all subjects, both genders and aged 18 years and older, who did not sustain a fracture during the study period. We randomly selected one control for each case, matched by gender and year of birth. The controls were selected using incidence-density sampling[17]. The date of the first fracture was used as index date for cases, and controls were assigned the index date of their matched case.

Fractures were classified into the following categories using ICD-10 codes: hip (S72.0-S72.2), radius/ulna (S52) and vertebrae (S12, S22.0-S22.1, S32.0-S32.2, S32.7, S32.8, T08). A major osteoporotic fracture was defined as a fracture of the hip, radius/ulna, vertebrae or humerus (S42.2-S42.4) according to the WHO definition[18].

Exposure of interest

We identified all drugs bought during the observation period using the Register of Medicinal Product Statistics. The dose of the drug was expressed as defined daily dose (DDD)[19]. ATC code A10B was used to determine exposure to non-insulin anti-diabetic drugs (NIAD), and patients were classified as current (1-91 days) or past (over 91 days) NIAD users, based on the time of the most recent prescription before the index date. Current NIAD users were divided into two mutually exclusive categories: never incretin users and incretin users.

To control for the potential that diabetes might act as a confounder[2], we used never incretin use (i.e. current NIAD use excluding incretin use) as the reference category in our analysis. ATC codes A10BX04 and A10BX07 were used to determine GLP1-RA exposure before the index date in the prescription database. Based on the time since the most recent prescription, cases and controls were classified as current (1-91 days), recent (92-182) or past (over 182 days) GLP1-RA users. The DDDs estimated the cumulative dose of GLP1-RAs for current GLP1-RA users, expressed as exenatide equivalents. The average daily dose was estimated by dividing the cumulative exposure

by the treatment time (time between the first GLP1-RA prescription and the index date).

Potential confounders

A history of the following potential confounders ever before the index date were taken into account: chronic obstructive pulmonary disease, previous fracture, rheumatoid arthritis, hypothyroidism, hyperthyroidism, cancer, retinopathy, alcoholism, secondary osteoporosis (diabetes type 1, hypogonadism or premature menopause), and congestive heart failure. The potential confounders were identified from the National Hospital register using ICD-10 and ICD-8 codes. Additional potential confounders included a prescription in the six months before the index date of the following drugs: dipeptidyl peptidase 4 inhibitors (DPP4-Is), oral glucocorticoids[20], lipid-modifying drugs, antidepressants[21], anxiolytics, hypnotics[22], antipsychotics, anti-Parkinson drugs[23], antihypertensives (beta-blockers, thiazide diuretics, renin angiotensin aldosterone system inhibitors, calcium channel blockers, and loop diuretics), and antiarrhythmics. The prescription database was used to explore the presence of a prescription of the above mentioned drugs.

Statistical analysis

Conditional logistic regression estimated the association between the use of GLP1-RAs versus use of other anti-hyperglycaemic drugs and risk of fracture, using SAS 9.3 software. Analyses were stratified by age, gender, type of fracture and for current GLP1-RA use also by average daily dose and cumulative exposure. The stratified analyses were determined a-priori, therefore no interaction analyses were performed. As a sensitivity analysis we adjusted for current metformin use, as it has been associated with a decreased risk of fracture[6]. As a second sensitivity analyses we adjusted the final model for insulin use, as insulin use has been associated with an increased fracture risk[5]. Final regression models were determined by stepwise backward elimination using a significance level of 0.05. All results are presented as odds ratios (ORs) with corresponding 95% confidence intervals (CIs).

Results

The study population consisted of 229,145 cases and the same number of controls. The mean age was 55 years old, and 56% were women. Baseline characteristics are shown in Table 2.5.1. Among cases we identified 6,993 (3.1%) current NIAD users (excluding incretin users) and 255 (0.1%) GLP1-RA users (current, recent or past). Similarly, we identified 7,209 (3.1%) NIAD users (excluding incretin users), and 220 (0.1%) GLP1-RA

users among controls. The mean duration of actual GLP1-RA use (from first GLP1-RA prescription till index date) was 36 weeks.

Table 2.5.1 Baseline characteristics[a].

Characteristic	Cases (N=229,145)	Controls (N=229,145)
Women	127,449 (55.6)	127,449 (55.6)
Mean age at index date (years, SD)	55 (20.6)	55 (20.6)
18 - 49 years	90,598 (39.5)	90,607 (39.5)
50 - 59 years	37,247 (16.3)	37,191 (16.2)
60 - 69 years	38,751 (16.9)	38,805 (16.9)
70 - 79 years	28,950 (12.6)	28,931 (12.6)
80+ years	33,599 (14.7)	33,611 (14.7)
History of comorbidities		
Type 1 diabetes mellitus	2,113 (0.9)	1,419 (0.6)
Alcoholism	11,147 (4.9)	4,824 (2.1)
Fracture	46,446 (20.2)	15,418 (6.7)
Hyperthyroidism	3,715 (1.6)	3,688 (1.6)
Hypothyroidism	2,887 (1.3)	2,496 (1.1)
COPD	10,812 (4.7)	7,418 (3.2)
Congestive heart failure	7,141 (3.1)	5,424 (2.4)
Cancer	21,893 (9.6)	18,486 (8.1)
Rheumatoid arthritis	3,912 (1.7)	2,795 (1.2)
Retinopathy	3,105 (1.4)	2,314 (1.0)
Neuropathy	7,915 (3.5)	6,093 (2.7)
Secondary osteoporosis	5,284 (2.3)	3,352 (1.5)
Drug use within six months before index date		
Anti-hyperglycaemic drugs	8,541 (3.7)	8,676 (3.8)
Biguanides	6,223 (2.7)	6,678 (2.9)
Sulphonylurea derivatives	3,900 (1.7)	3,809 (1.7)
Thiazolidinediones	262 (0.1)	183 (0.1)
Glinides	87 (0.0)	83 (0.0)
GLP1-RAs	255 (0.1)	220 (0.1)
DPP4-Is	643 (0.3)	707 (0.3)
Insulins	4,900 (2.1)	3,261 (1.4)
Short acting	2,046 (0.9)	1,139 (0.5)
Intermediate acting	2,049 (0.9)	1,411 (0.6)
Long acting	1,444 (0.6)	869 (0.4)
Combinations	1,679 (0.7)	1,200 (0.5)
Statins	31,874 (13.9)	32,064 (14.0)
Antiarrhythmics	818 (0.4)	522 (0.2)
Beta blockers	23,281 (10.2)	23,592 (10.3)
Thiazide diuretics	20,425 (8.9)	21,547 (9.4)
RAAS inhibitors	37,555 (16.4)	39,379 (17.2)
Calcium channel blockers	22,942 (10.0)	22,816 (10.0)
Loop diuretics	16,905 (7.4)	12,766 (5.6)
Antidepressants	33,644 (14.7)	20,338 (8.9)
Anti-Parkinson drugs	3,174 (1.4)	1,814 (0.8)
Antipsychotics	8,032 (3.5)	4,867 (2.1)
Anxiolytics	14,668 (6.4)	10,431 (4.6)
Hypnotics	19,137 (8.4)	14,332 (6.3)
Glucocorticoids	9,390 (4.1)	6,858 (3.0)
Bisphosphonates	7,371 (3.2)	4,913 (2.1)

Table 2.5.1 (continued)

Characteristic	Cases (N=229,145)	Controls (N=229,145)
Raloxifene	214 (0.1)	148 (0.1)
Vitamin D	211 (0.1)	169 (0.1)
Calcium	1,885 (0.8)	1,375 (0.6)
Strontium ranelate	194 (0.1)	101 (0.0)
Parathyroid hormone	130 (0.1)	70 (0.0)
Calcitonin	2 (0.0)	1 (0.0)
Hormone replacement therapy	11,912 (5.2)	13,955 (6.1)
Beta2-agonists	10,436 (4.6)	8,130 (3.6)
Inhaled anticholinergics	4,569 (2.0)	3,277 (1.4)
Inhaled corticosteroids	5,265 (2.3)	4,657 (2.0)

COPD: chronic obstructive pulmonary disease; DPP4-I: dipeptidyl peptidase 4 inhibitor; GLP1-RA: glucagon-like peptide 1 receptor-agonist; RAAS: renin angiotensin aldosterone system; SD: Standard deviation. [a] Data are number (%) of patients, unless stated otherwise.

Current GLP1-RA use was not associated with a decrease in fracture risk: adjusted (adj.) OR: 1.16 (95% CI: 0.83-1.63). Similarly, no significant decrease was observed for recent use (adj. OR 1.03, 95% CI: 0.69-1.53), or for past use (adj. OR 1.09, 95% CI: 0.82-1.46). Having no prior use of NIAD (never use) was associated with a significant increase in risk of fracture (adj. OR 1.10, 95% CI: 1.06-1.14), as well as past NIAD use (adj. OR 1.12, 95% CI: 1.05-1.20). The risk of fracture was not reduced after stratification by sex or age, Table 2.5.2.

Table 2.5.3 shows that current GLP1-RA use was not associated with osteoporotic fracture risk (adj. OR 0.78, 95% CI: 0.44-1.39). We identified similar trends for recent GLP1-RA use (adj. OR 0.97, 95% CI: 0.52-1.79) and for past use (adj. OR 0.94, 95% CI: 0.60-1.48). Stratification by sex and age did not substantially change the results (data not shown). There was no significant association with fracture risk of the radius/ulna for current GLP1-RA use (adj. OR 0.93, 95% CI: 0.48-1.80), recent GLP1-RA use (adj. OR 1.32, 95% CI: 0.55-3.17), or for past GLP1-RA use (adj. OR 0.93, 95% CI: 0.48-1.80). Stratification by gender and age did not show a decreased fracture risk (data not shown). Hip and vertebral fracture risk was not associated with GLP1-RA use (current, recent or past), Table 2.5.3.

The risk of any fracture was comparable, and not significantly reduced, among the cumulative and average daily dose groups, Table 2.5.4. The adj. OR for the highest cumulative dose (≥5.5 mg exenatide equivalent) was 0.98 (95% CI: 0.50-1.94) and the adj. OR for the highest average daily dose (≥22.5 mcg exenatide equivalent per day) was 1.66 (95% CI: 0.86-3.19). When only osteoporotic fractures were considered, there was again, no association with a decreased risk of fracture. Adj. OR for the highest cumulative dose (≥5.5mg exenatide equivalent) was 0.93 (95% CI: 0.30-2.92) and the adj. OR for the highest average daily dose (≥22 mcg exenatide equivalent per day) was 1.40 (95% CI: 0.47-4.13).

Table 2.5.2 Use of GLP1-RAs and risk of any fracture.

Exposure	No. of cases (N=229,145)[a]	No. of controls (N=229,145)[a]	Crude OR (95% CI)	Adjusted OR (95% CI)[b]
Never NIAD use	217,623	218,194	1.03 (0.99 - 1.06)	1.10 (1.06 - 1.14)*
Past NIAD use	3,631	2,815	1.33 (1.25 - 1.41)*	1.12 (1.05 - 1.20)*
Current NIAD use excluding incretin use	6,993	7,209	Reference	Reference
Past GLP1-RA use (183 - 365 days before index date)	120	93	1.33 (1.01 - 1.74)*	1.09 (0.82 - 1.46)
Recent GLP1-RA use (92 - 182 days before the indexdate)	55	56	1.01 (0.70 - 1.47)	1.03 (0.69 - 1.53)
Current GLP1-RA use (1 - 91 days before the indexdate)	80	71	1.16 (0.84 - 1.60)	1.16 (0.83 - 1.63)
By sex				
Males	38	39	0.98 (0.63 - 1.54)	0.97 (0.60 - 1.57)
Females	42	32	1.37 (0.86 - 2.18)	1.43 (0.87 - 2.32)
By age on index date				
<50 years	12	10	1.28 (0.52 - 3.11)	1.19 (0.47 - 2.98)
50 - 59 years	20	22	0.95 (0.51 - 1.75)	0.99 (0.51 - 1.89)
60 - 69 years	33	26	1.09 (0.64 - 1.86)	1.26 (0.71 - 2.24)
70+ years	15	13	1.29 (0.60 - 2.77)	1.04 (0.46 - 2.35)

CI: confidence interval; DPP4-I: dipeptidyl peptidase 4 inhibitor; GLP1-RA: glucagon-like peptide 1 receptor agonist; NIAD: non-insulin anti-diabetic-drug; OR: odds ratio. Never NIAD use: no NIAD prescription before the index date. Past NIAD use: most recent NIAD prescription over 91 days before index date. Current NIAD use: most recent NIAD prescription within 91 days before index date. * Statistically significant, (P<0.05). [a] The numbers do not add up precisely to the total number of fractures because DPP4-I exposure is not shown. [b] Adjusted for history of cancer, chronic obstructive pulmonary disease, fracture, alcoholism, rheumatoid arthritis, secondary osteoporosis, hyperthyroidism, retinopathy, neuropathy, heart failure and use of DPP4-Is, glucocorticoids, statins, anxiolytics, hypnotics, antidepressants, antipsychotics, anti-Parkinson drugs, beta-blockers, thiazide diuretics, renin angiotensin aldosterone system inhibitors, loop diuretics and antiarrhythmics.

Table 2.5.3 Use of GLP1-RAs and fracture risk at different skeletal sites.

Fracture sites	No. of cases[a]	No. of controls[a]	Crude OR (95% CI)	Adjusted OR (95% CI)
Osteoporotic fracture	96,774	96,774	-	-
Never NIAD use	90,297	90,677	1.02 (0.97 - 1.07)	1.07[a] (1.02 - 1.13)*
Current NIAD use excl. incretin use	3,957	4,058	Reference	Reference
Past GLP1-RA use (>182 days before the indexdate)	47	41	1.17 (0.77 - 1.78)	0.94[a] (0.60 - 1.48)
Recent GLP1-RA use (92 - 182 before the indexdate)	23	24	0.98 (0.55 - 1.73)	0.97[a] (0.52 - 1.79)
Current GLP1-RA use (0 - 91 days before the indexdate)	23	31	0.76 (0.44 - 1.30)	0.78[a] (0.44 - 1.39)
Hip	24,328	24,328	-	-
Never NIAD use	21,923	22,273	0.94 (0.87 - 1.01)	1.00[b] (0.92 - 1.09)
Current NIAD use excl. incretin use	1,454	1,388	Reference	Reference
Past GLP1-RA use (>182 days before the indexdate)	9	4	2.11 (0.65 - 6.86)	1.74[b] (0.47 - 6.49)
Recent GLP1-RA use (92 - 182 before the indexdate)	5	4	1.18 (0.32 - 4.40)	1.34[b] (0.26 - 6.93)
Current GLP1-RA use (0 - 91 days before the indexdate)	4	4	0.99 (0.25 - 3.97)	0.77[b] (0.16 - 3.73)
Radius/ulna	47,905	47,905	-	-
Never NIAD use	45,848	45,423	1.29 (1.19 - 1.39)*	1.29[c] (1.19 - 1.40)*
Current NIAD use excl. incretin use	1,273	1,617	Reference	Reference
Past GLP1-RA use (>182 days before the indexdate)	19	21	1.16 (0.62 - 2.16)	0.93[c] (0.48 - 1.80)
Recent GLP1-RA use (92 - 182 before the indexdate)	11	12	1.18 (0.52 - 2.68)	1.32[c] (0.55 - 3.17)
Current GLP1-RA use (0 - 91 days before the indexdate)	11	20	0.70 (0.34 - 1.47)	0.93[c] (0.48 - 1.80)
Vertebral	9,004	9,004	-	-
Never NIAD use	8,396	8,450	1.01 (0.87 - 1.17)	1.11[d] (0.93 - 1.32)
Current NIAD use excl. incretin use	364	369	Reference	Reference
Past GLP1-RA use (>182 days before the indexdate)	4	5	0.80 (0.21 - 3.02)	0.97[d] (0.22 - 4.32)
Recent GLP1-RA use (92 - 182 before the indexdate)	2	4	0.50 (0.09 - 2.75)	0.30[d] (0.05 - 1.84)
Current GLP1-RA use (0 - 91 days before the indexdate)	2	2	1.00 (0.14 - 7.15)	2.02[d] (0.28 - 14.75)

CI: confidence interval; COPD: chronic obstructive pulmonary disease; DPP4-I: dipeptidyl peptidase 4 inhibitor; GLP1-RA: glucagon-like peptide 1 receptor agonist; NIAD: non-insulin anti-diabetic-drug; OR: odds ratio; RAAS: renin angiotensin aldosterone system. Never NIAD use: no NIAD prescription before the index date. Current NIAD use: most recent NIAD prescription within 91 days before index date. * Statistically significant, (P<0.05). [a] The numbers do not add up precisely to the total number of fractures because past NIAD use and DPP4-I exposure are not shown. [b] Adjusted for (f) and history of retinopathy, heart failure, COPD and use of glucocorticoids, statins, anxiolytics, antipsychotics, beta-blockers, thiazide diuretics, RAAS inhibitors and antiarrhythmics. [c] Adjusted for (f) and history of retinopathy, heart failure, COPD and use of statins, anxiolytics, RAAS inhibitors, antiarrhythmics, glucocorticoids and antipsychotics. [d] Adjusted for (f) and use of beta-blockers, thiazide diuretics, RAAS inhibitors and calcium channel inhibitors. [e] Adjusted for (f) and history of COPD and use of glucocorticoids, anxiolytics, RAAS inhibitors and antipsychotics. [f] History of cancer, alcoholism, fracture, rheumatoid arthritis and secondary osteoporosis and use of DPP4-Is, antidepressants, anti-Parkinson drugs, loop diuretics, and hypnotics.

Table 2.5.4 Use of GLP-1 RAs and risk of any fracture stratified by cumulative and average daily dose.

	Any Fracture			Osteoporotic fracture		
	No. of cases (N=229,145)[a]	No. of controls (N=229,145)[a]	Adjusted OR (95% CI)[b]	No. of cases (N=96,774)[a]	No. of controls (N=96,774)[a]	Adjusted OR (95% CI)[c]
Current NIAD use excl. incretins	6,993	7,209	Reference	3,957	4,058	Reference
Current GLP1-RA use	80	71	1.16 (0.83 - 1.63)	23	31	0.78 (0.44 - 1.39)
By cumulative exposure[d]						
0 - 1.4 mg	36	34	1.05 (0.63 - 1.73)	10	17	0.64 (0.28 - 1.47)
1.4 - 2.7 mg	14	11	1.60 (0.69 - 3.69)	3	5	0.57 (0.12 - 2.74)
2.7 - 5.5 mg	12	8	1.55 (0.60 - 3.95)	4	2	2.05 (0.32 - 13.30)
≥5.5 mg	18	18	0.98 (0.50 - 1.94)	6	7	0.93 (0.30 - 2.92)
By average daily dose[d]						
<15 mcg/day	16	19	0.91 (0.47 - 1.84)	3	9	0.35 (0.09 - 1.39)
15 - 22.49 mcg/day	38	36	1.07 (0.66 - 1.73)	12	15	0.78 (0.35 - 1.75)
≥22.5 mcg/day	26	16	1.66 (0.86 - 3.19)	8	7	1.40 (0.47 - 4.13)
Recent GLP1-RA use	55	56	1.03 (0.69 - 1.53)	23	24	0.97 (0.52 - 1.79)
Past GLP1-RA use	120	93	1.09 (0.82 - 1.46)	47	41	0.94 (0.60 - 1.48)

CI: confidence interval; DPP4-I: dipeptidyl peptidase 4 inhibitor; GLP1-RA: glucagon-like peptide 1 receptor agonist; NIAD: non-insulin anti-diabetic-drug; OR: odds ratio. * Statistically significant, (P<0.05). [a] The numbers do not add up precisely to the total number of fractures because never NIAD, past NIAD and DPP4-I use are not shown. [b] Adjusted for history of cancer, alcoholism, chronic obstructive pulmonary disease, fracture, rheumatoid arthritis, hyperthyroidism, secondary osteoporosis, retinopathy, neuropathy and heart failure and use of DPP4-Is, glucocorticoids, statins, antidepressants, anxiolytics, hypnotics, antipsychotics, anti-Parkinson drugs, beta-blockers, thiazide diuretics, renin angiotensin aldosterone system inhibitors, loop diuretics, antiarrhythmics. [c] Adjusted for (b), but not for hyperthyroidism and neuropathy. [d] In exenatide equivalents.

Adjusting the main analysis for current metformin use did not substantially change our results. The adj. OR for current GLP1-RA use was 1.17 (95% CI: 0.83-1.64), for recent GLP1-RA 1.03 (95% CI: 0.70-1.54) and for past GLP1-RA use 1.09 (95% CI: 0.82-1.46). As a second sensitivity analysis we additionally adjusted the main analysis for insulin use, this did not alter the results. The adj. OR for current GLP1-RA use was 1.13 (95% CI: 0.80-1.58), for recent use 1.00 (95% CI: 0.67-1.49) and for past use 1.05 (95% CI: 0.79-1.41).

Discussion

The present study showed that current GLP1-RA use was not associated with a decreased risk of any fracture, as compared to use of other anti-hyperglycaemic drugs, and current GLP1-RA use was not associated with a reduced risk of other fracture types. Moreover, stratification of current GLP1-RA by cumulative or average daily dose was not associated with a decreased risk of fracture.

The results of the present study are in line with the results of a meta-analysis of clinical trials on the effect of GLP1-RAs on fracture risk, which showed that fracture risk was not significantly reduced with use of GLP1-RAs[12,24]. Our results are also in keeping with a large clinical trial (n=16,492) on the effect of a DPP4-I, saxagliptin, which showed no difference in risk of fracture with use of DPP4-I use and placebo[25]. Additionally, the present results are also supported by the results of a cohort study which was not able to show a decreased risk of fracture with use of GLP1-RAs as compared to use of other anti-hyperglycaemic drugs[13] and a cohort study that compared use of DPP4-I to use of other anti-hyperglycaemic drugs[26]. The pathway by which DPP4-Is might affect bone metabolism may be the same as that of GLP1-RAs because DPP4-Is inhibit the degradation of GLP1[2]. Moreover, our results are indirectly supported by a clinical trial on the effect of a GLP1-RA, exenatide, on markers of bone metabolism[27], which reported that bone markers were unaffected after 44 weeks of exenatide treatment.

We acknowledge that our findings are in contrast to those of a meta-analysis of randomised clinical trials (n=22,055) that showed a significant 40% reduction of fracture risk with the use of DPP4-Is[28] as compared to active treatment or placebo. However, studies that were included in this meta-analysis did not routinely collect fractures as an outcome of interest, the number of fractures was low and different comparator groups had been used[27]. The results from this meta-analysis should likely be interpreted with caution as the low number of events in a meta-analysis of adverse events can give biased estimates[28,29].

Results of in vitro studies have suggested that use of GLP1-RAs might have a beneficial effect on bone metabolism[7-9], yet different mechanisms of effect have been hypothesized. In vitro studies have shown that GLP1 receptors are present on bone marrow stromal cells[30], immature osteoblast[31] and osteocytes[10], and binding of GLP1 to

its receptor on bone cells leads to increased osteoblast activity[9], and decreased osteoclast activity[10]. This could then lead to increased bone formation and a reduced fracture risk. However, more research is needed to assess whether GLP1-RAs are also able to bind to GLP1 receptors on human bone cells and whether this could ultimately result in a reduced risk of fracture.

An unexpected finding was that never and past NIAD use showed an increased fracture risk as compared to current use of other anti-hyperglycaemic drugs. This may have been the result of residual confounding by high body mass index (BMI), which has been shown to decrease fracture risk[32]. T2DM has been associated with an increased BMI and therefore it might be that the increased risk of never NIAD use is a result of a higher BMI in the current NIAD use group which was used as a reference group. The past NIAD use group included patients with T2DM who use insulin only. Insulin use has been associated with a two-fold increased risk of fracture as compared to patients with T2DM who use NIADs[2].

There are several strengths to the current study. First the use of a nationwide population register permitted the examination of a large number of cases and controls. The data used were also collected longitudinally, and for prescriptions this permitted us to calculate a reliable cumulative and average daily dose. Second, the data used to identify fractures has been validated[14]. Third, we were able to adjust our analyses for many potential confounders. When interpreting our results, we are mindful of a couple of limitations. We were not able to adjust for potential confounders such as BMI, glycosylated hemoglobin type A1c (HbA1c), smoking, amount of exercise and serum vitamin D levels. A high BMI has been associated with a reduced risk of fracture[32] and therefore a decreased risk of fracture with GLP1-RA use could have represented the association between high BMI and fracture risk. Nevertheless, we did not show a reduced fracture risk with GLP1-RA use. Smoking is a risk factor of fracture[33], but there is no evidence that GLP1-RA users have different smoking behaviors than patients treated with other anti-hyperglycaemic drugs. Exercise has been associated with a decreased risk of fracture[34], GLP1-RA users might perform less exercise which could result in an overestimation of the effect. Lower levels of serum vitamin D have been associated with more severe T2DM complications and increased fracture risk[35,36]. Not adjusting for serum vitamin D levels might lead to an overestimation of the effect when GLP1-RA users have lower serum vitamin D levels as compared to users of other anti-hyperglycaemic drugs. Thus, while we were able to identify a large number of confounders due to the completeness of the registry data, it is acknowledged that some residual confounding might still be present. In addition, we tried to capture severity of diabetes by correcting our analyses for known complications of diabetes, such as neuropathy and retinopathy. Moreover we used current NIAD use (excluding incretin use) as a reference group, because diabetes itself might act as a confounder. Current NIAD use included use of TZDs or insulins, which have been shown to increase fracture risk[3,4]. The result of this bias in the reference category may be an observed

artificial inverse association between GLP1-RA use and risk of fracture which could have falsely supported our hypothesis. However, we did not observe an inverse association between GLP1-RA use and risk of fracture. Current NIAD use also included metformin use which has been associated with a reduced fracture risk and therefore it might mask a decreased association between GLP1-RA use and fracture risk. Nevertheless, statistical adjustment of the main analysis for current metformin use did not alter the results.

Although the total number of fractures was high, the number of some fracture types (i.e., hip and radius/ulna) was not high enough to stratify current GLP1-RA use by cumulative and average dose, and to keep adequate statistical power. In the analyses with hip and vertebral fracture as outcome the number of GLP1-RA users was quite small, therefore these results should be interpreted with caution. The average duration of actual GLP1-RA use (36 weeks) in the present study was rather short, which might be the reason that we were unable to observe an association between GLP1-RA use and fracture risk. However, after stratification of current GLP1-RA use by cumulative dose, risk of fracture was not decreased in the group with patients who had used on average 15 microgram exenatide equivalent per day for at least one year (cumulative dose: ≥5.5 mg exenatide equivalent).

In conclusion, we showed in a population-based case-control study that the use of GLP1-RAs (current, recent or past) is not associated with fracture risk as compared to use of other anti-hyperglycaemic drugs. In addition, current GLP1-RA use stratified by cumulative and average daily dose was not associated with a decreased fracture risk. More research is needed, and in particular future studies should focus on the effect of long-term use of GLP1-RAs on fracture risk.

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2.5

Chapter 2.6

The use of incretins and fractures – a meta-analysis on population-based real life data

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Br J Clin Pharmacol. 2017;83(4):923-926

Abstract

Introduction

The aim of the present study was to estimate the effect of use of incretins on fracture risk in the real world situation by a meta-analysis of the available population-based cohort data.

Methods

Pubmed and Embase were searched for original articles investigating use of incretin agents, and fracture risk up to December 2015. Adjusted results were extracted and results were pooled by use of generic inverse variance methods, assuming a random-effects model.

Results

Neither current dipeptidyl peptidase 4 inhibitor use, nor current glucagon-like peptide 1 receptor agonist use was associated with a decreased risk of fracture: pooled relative risk [pooled RR (95% CI): 1.02 (0.91-1.13) and 1.03 (0.87-1.22)], respectively.

Conclusion

This meta-analysis demonstrated that current use of incretin agents, was not associated with decreased fracture risk. Our findings show the value of representative real-world populations, and the risks associated with suggesting benefits for medications on the basis of safety reporting in randomised controlled trials.

Introduction

Fractures are associated with increased morbidity and mortality, and place a considerable economic burden upon health care systems[1]. Patients with type 2 diabetes mellitus (T2DM) are at increased fracture risk, both from the disease itself and potentially from associated medications[2]. Since 2007, incretin agents, such as dipeptidyl peptidase 4 inhibitors (DPP4-Is) and glucagon-like peptide 1 receptor agonists (GLP1-RAs), have been available for the treatment of T2DM.

Interestingly, a meta-analysis, solely based upon randomised controlled trial (RCT) data, showed that the use of DPP4-Is was associated with a 40% reduction in fracture risk[3]. In contrast, two meta-analyses of RCT data comparing GLP1-RAs to active comparators and/or placebo showed no association between GLP1-RA use and fracture risk[4,5]. Nevertheless, stratification by type of GLP1-RA resulted in a 62% reduced risk with a specific GLP1-RA, while another type showed a two-fold increased risk[5].

We have recently investigated the association between incretin use and fracture risk, using real-life data from large population-based cohorts[6-9]. In contrast to the RCT meta-analyses[3,5] we did not observe a reduced fracture risk with use of either of the incretins.

The aim of the present study was to obtain the highest quality estimate of the effect of incretins on fracture risk in the real world situation by meta-analysis of the available population-based cohort data.

Methods

Pubmed and Embase were searched for original published and peer-reviewed articles investigating incretin agents, either DPP4-Is or GLP1-RAs, and fracture risk up to 2015. The following search string was used: "(dpp-4 inhibitors or dipeptidyl peptidase-4 inhibitors or Sitagliptin or Vildagliptin or Saxagliptin or Linagliptin or Anagliptin or Teneligliptin or Alogliptin or Trelagliptin or Gemigliptin or Dutogliptin or Omarigliptin or exenatide or liraglutide or lixisenatide or albiglutide or dulaglutide or GLP1-analogues or glucagon-like peptide 1 or incretin) and fracture". Two authors (JD and FV) reviewed the titles and abstracts of the potential eligible articles. To be included, a study had to meet the following criteria: (1) the use of an observational study design; (2) a comparison of the use of at least one of the incretin agents (DPP4-I or GLP1-RA) to use of other non-insulin anti-diabetic drugs (NIADs); (3) report fracture as outcome; (4) reporting relative risks (RRs), odds ratios (ORs), or hazard ratios (HRs) including 95% confidence intervals (CIs); (5) reporting in English. References of included articles were searched to identify additional articles.

The name of first author, the year of publication, the study design, the number of patients per exposure group, the number of events, the duration of follow-up and the point estimates with corresponding 95% CIs were extracted from the included studies.

Data on specified fracture sites and results stratified by age and gender were extracted as well. Additionally, data on average daily dose en cumulative dose were extracted, if the average and cumulative daily dose categories were not similar between studies, authors were contacted to provide adjusted results.

Results were pooled by use of generic inverse variance methods, assuming a random effects model. Sub-analyses were performed for different fracture sites (hip, radius/ulna, clinical vertebral and major osteoporotic fracture), average daily dose, cumulative dose, different age categories and sex. Heterogeneity was assessed by the Cochrane Q-statistic and the I2 statistic. Publication bias could not be assessed due to the low number of included studies per exposure (n=2)[10]. Analyses were performed using RevMan Version 5.3 (Cochrane Collaboration, Oxford, UK). A p-value <0.05 was considered to be statistically significant.

Results

The initial electronic search resulted in a total of 125 hits (Pubmed: 59; Embase 66), Figure 2.6.1. After screening of the abstracts and titles a total of four studies met our inclusion criteria. Two of them were cohort studies whereas the other two were case-control studies[6-9]. These studies contained in total data on 22,961 current DPP4-I users (568 fractures) and 8,505 current GLP1-RA users (202 fractures), Table 2.6.1.

We found that neither current DPP4-I use, nor current GLP1-RA use was associated with a decreased risk of fracture (corresponding forest plots Figure 2.6.2 and Figure 2.6.3): pooled relative risk [pooled RR (95% CI): 1.02 (0.91-1.13) and 1.03 (0.87-1.22)] respectively, except for GLP1-RA use which was associated with an increased risk of vertebral fracture risk [pooled RR (95% CI) 1.86 (1.19-2.91)] (data not shown). For both DPP4-I use and GLP1-RA use no heterogeneity was found across studies (DPP4-I: Q-statistic: 0.28 (p-value=0.60), I^2=0%; GLP1-RA: 0.66, Q-statistic: (p-value 0.42), I^2=0%, Figure 2.6.2 and 2.6.3, respectively).

The results were similar if DPP4-I use was stratified according to cumulative exposure or average daily dose. When GLP1-RA use was stratified according to cumulative exposure or average daily dose there was no consistent increased or decreased fracture risk with cumulative dose, whereas fracture risk was increased if the average daily GLP1-RA dose exceeded 22.5 mcg/day [pooled RR (95% CI) 1.63 (1.11-2.41)], all Table 2.6.2.

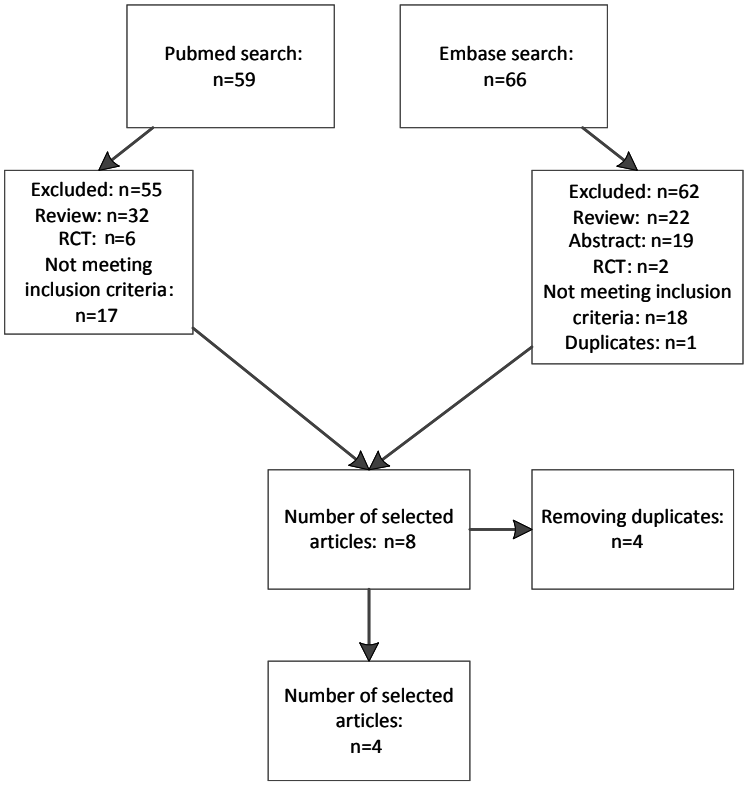

Figure 2.6.1 Flowchart of included studies. RCT: randomised controlled trial.

Table 2.6.1 Characteristics of included studies.

Author and year [Reference]	Investigated exposure	Study design	Number of patients exposed to incretin agents	Number of patients in the reference group	Characteristics at baseline
Driessen et al. 2014 [6]	DPP4-I	Cohort study	22,510	194,306	Mean age: 61 % Women: 47.3
Driessen et al. 2015 [8]	DPP4-I	Case-control study	Cases: 219 Controls: 232	Cases: 6993 Controls: 7209	Mean age: 55 % Women: 55.6
Driessen et al. 2015 [7]	GLP1-RA	Cohort study	8,354	208,462	Mean age: 61 % Women: 47.3
Driessen et al. 2015 [9]	GLP1-RA	Case-control study	Cases:80 Controls:71	Cases:6993 Controls:7209	Mean age: 55 % Women: 55.6

DPP4-I: dipeptidyl peptidase 4 inhibitor; GLP1-RA: glucagon-like peptide 1 receptor agonist.

Study or subgroup	Log [Risk Ratio]	SE	Weight	Risk Ratio IV, Random, 95% CI	Risk Ratio IV, Random, 95% CI
Driessen et al. 2014 [6]	0.03	0.06	84.5%	1.03 [0.92, 1.16]	
Driessen et al. 2015 [8]	-0.05	0.14	15.5%	0.95 [0.72, 1.25]	
Total (95% CI)			**100%**	**1.02 [0.91, 1.13]**	
Heterogeneity; Chi^2 = 0.28, df =1 (P=0.60); I^2 = 0%					
Test for overall effect: Z = 0.32 (P = 0.75)					

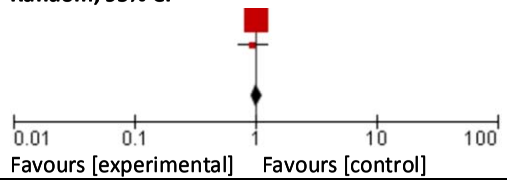

Figure 2.6.2 Forest plots of current DPP4-I use compared to current other NIAD use. CI: confidence interval; df: degrees of freedom; DPP4-I: dipeptidyl peptidase 4 inhibitor; IV: inverse variance; NIAD: non-insulin anti-diabetic drug; SE: standard error.

Study or subgroup	Log [Risk Ratio]	SE	Weight	Risk Ratio IV, Random, 95% CI	Risk Ratio IV, Random, 95% CI
Driessen et al. 2015 [7]	-0.01	0.1	74.3%	0.99 [0.81, 1.20]	
Driessen et al. 2015 [9]	0.15	0.17	25.7%	1.16 [0.83, 1.62]	
Total (95% CI)			**100%**	**1.03 [0.87, 1.22]**	
Heterogeneity; Chi^2 = 0.66, df =1 (P=0.42); I^2 = 0%					
Test for overall effect: Z = 0.36 (P = 0.72)					

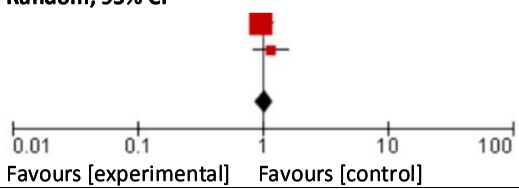

Figure 2.6.3 Forest plots of current GLP1-RA use compared to current other NIAD use. CI: confidence interval; df: degrees of freedom; GLP1-RA: glucagon-like peptide 1 receptor agonist; IV: inverse variance; NIAD: non-insulin anti-diabetic drug; SE: standard error.

Table 2.6.2 Risk of fracture in DPP4-I and GLP1-RA users compared with NIAD users, by age, sex, cumulative exposure and average daily dose.

DPP4-I		GLP1-RA	
	Pooled RR (95% CI)		Pooled RR (95% CI)
Risk of any fracture		Risk of any fracture	
Current NIAD use	Reference	Current NIAD use	Reference
excl. incretin use		excl. incretin use	
Current DPP4-I use	1.02 (0.91 - 1.13)	Current GLP1-RA use	1.03 (0.87 - 1.22)
By sex		By sex	
Males	1.01 (0.86 - 1.17)	Males	1.00 (0.79 - 1.27)
Females	0.99 (0.80 - 1.23)	Females	1.11 (0.76 - 1.61)
By age		By age	
<50 years	0.82 (0.56 - 1.20)	<50 years	0.86 (0.58 - 1.27)
50 - 59 years	1.18 (0.95 - 1.47)	50 - 59 years	0.84 (0.62 - 1.15)
60 - 69 years	1.01 (0.85 - 1.20)	60 - 69 years	1.35 (1.06 - 1.73)*
70 - 79 years	1.08 (0.86 - 1.37)	70+ years	0.77 (0.49 - 1.22)
80+ years	0.98 (0.76 - 1.25)		
By cumulative exposure[a]		By cumulative exposure[b]	
0 - 9.1 g	0.98 (0.79 - 1.22)	0 - 1.3 mg	1.02 (0.75 - 1.37)
9.2 - 18.2 g	1.06 (0.84 - 1.33)	1.4 - 2.6 mg	1.18 (0.79 - 1.76)
18.3 - 36.4 g	0.93 (0.75 - 1.16)	2.7 - 5.4 mg	1.60 (1.04 - 2.47)*
≥36.5 g	1.01 (0.85 - 1.20)	≥5.5 mg	0.94 (0.59 - 1.51)
By average daily dose[a]		By average daily dose[b]	
<100 mg/day	1.08 (0.76 - 1.55)	<15.0 mcg/day	1.05 (0.80 - 1.37)
100 - 149 mg/day	0.92 (0.79 - 1.06)	15.0 - 22.4 mcg/day	0.91 (0.70 - 1.17)
≥150 mg/day	0.96 (0.80 - 1.14)	≥22.5 mcg/day	1.63 (1.11 - 2.41)*

CI: confidence interval; DPP4-I: dipeptidyl peptidase 4 inhibitor; GLP1-RA: glucagon-like peptide 1 receptor agonist; NIAD: non-insulin anti-diabetic drug; RR: relative risk. * Statistically significant, p<0.05. [a] In sitagliptin equivalents. [b] In exenatide equivalents.

Discussion

The results of this meta-analysis on real-life population-based data demonstrate that, contrary to pooled RCT-data[3,5], the current use of incretins (either DPP4-Is or GLP1-RAs) was not associated with a decreased fracture risk. Moreover, GLP1-RA use was associated with an increased risk of any fracture if the average daily dosage exceeded 22.5 mcg/day. The present results were in line with a previous meta-analysis, showing no association between use of GLP1-RA and risk of fracture[4]. It is possible that the discrepancies between the pooled RCT-data and our real-life population-based data may be a result of selection bias due to the use of strict inclusion and exclusion criteria with RCTs and the fact that data on fractures in the RCT studies were not predefined outcomes and therefore not routinely systematically collected. Importantly, the notion that incretins may have skeletal effects stems from in vitro and experimental animal studies, possibly acting via osteoclast inhibition and modulation of thyroid C-cells,

which express incretin receptors[11,12]; the translation of such observations to the human clinical situation must be viewed with caution.

A particular strength of this short report is, next to its analyses of real-life population-based data, its use of the same cumulative and average daily dose categories which allowed us to use the same definitions across the studies. When interpreting the results, a couple of limitations are worth mentioning. The number of fractures with current GLP1-RA use was relatively small, which limited the statistical power to detect associations, particularly when stratified by cumulative exposure, average daily dose and fracture type. In addition, only a small number of observational studies, all performed by us, could be included in the present meta-analysis. Another limitation is the relative short duration of incretin use (37 weeks to 1.7 years)[6-9]. In addition, the included studies adjusted for different sets of confounders, which may have biased our results. We nevertheless have tested the hypothesis that incretin use was associated with a decreased risk of fracture in multiple ways, and none of the analyses showed a decreased risk of fracture. Moreover we used data representative for the United Kingdom (2007-2012) and data on all fractures in Denmark between 2007 and 2011.

In short, this meta-analysis demonstrated that current use of incretin agents, either DPP4-Is or GLP1-RAs, was not associated with decreased fracture risk. Moreover, current GLP1-RA use was associated with an increased risk of any fracture when the average daily dosage exceeded 22.5 mcg/day. Our findings show the value of representative real world populations, and the risks associated with suggesting benefits for medications on the basis of safety reporting in RCTs. An adequately powered trial with fracture as the primary endpoint will be required to properly demonstrate the skeletal efficacy or otherwise of incretins.

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2.6

Chapter 2.7

Long-term use of dipeptidyl peptidase 4 inhibitors and risk of fracture; a retrospective population-based cohort study

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Abstract

Introduction

Patients with type 2 diabetes mellitus (T2DM) have an increased risk of fracture as compared to patients without T2DM. It has been suggested based on clinical trial data that use of dipeptidyl peptidase 4 inhibitors (DPP4-Is), an anti-hyperglycaemic drug, is associated with a decreased risk of fracture as compared to patients with and without T2DM. However, observational studies have failed to show this association, which might be due to the short duration of use. Therefore, the aim of the present study was to investigate the association between long-term DPP4-I use and risk of fracture.

Methods

A retrospective population-based cohort study, using data from the Clinical Practice Research Datalink (CPRD) database (2007-2015), was conducted. All patients (n=328,254) with at least one prescription for a non-insulin anti-diabetic drug (NIAD), aged 18+ during data collection, were included. Cox proportional hazards models were used to estimate the hazard ratio of any, osteoporotic and hip fracture in DPP4-I users versus other NIAD users. Analyses were stratified by continuous duration of DPP4-I use. Time-dependent adjustments were made for age, sex, life-style, comorbidity and concomitant drug use.

Results

Current use of DPP4-Is was not associated with risk of any fracture (adjusted (adj.) hazard ratio (HR): 0.99 (95% confidence interval (CI) 0.93-1.06)) as compared to current other NIAD use. Current use of DPP4-Is was also not associated with risk of osteoporotic or hip fracture. After stratification by continuous duration of DPP4-I use the highest category was not associated with any (>4.0-8.5 years of DPP4-I use; adj. HR: 0.99 (0.70-1.41)), osteoporotic (>3.0-8.5 years of DPP4-I use; adj. HR: 0.75 (0.52-1.09)), or hip (>2.0-8.5 years of DPP4-I use; adj. HR: 1.24 (0.85-1.79)) fracture.

Conclusion

Continuous long-term DPP4-I use (defined as >4.0-8.5 years of DPP4-I use for any fracture, >3.0-8.5 years for osteoporotic fracture and >2.0-8.5 years for hip fracture, respectively) was not associated with risk of any, osteoporotic, or hip fracture. These findings may be of value for clinical decisions regarding treatment of T2DM patients, especially those at high fracture risk.

Introduction

Worldwide, about 422 million people suffer from type 2 diabetes mellitus (T2DM)[1]. Besides other complications T2DM has been associated with an increased risk of fracture compared to subjects without T2DM[2]. Explanations for this increased fracture risk include an increased risk of falling[3], the pathophysiology of diabetes itself on bone quality[4] as well as the effect of anti-hyperglycaemic drugs used in T2DM[5]. Dipeptidyl peptidase 4 inhibitors (DPP4-Is), are a relatively new type of anti-hyperglycaemic drugs which have been marketed since 2006[6].

It has been suggested that DPP4-Is might influence bone metabolism and thereby potentially reduce fracture risk[5]. A first meta-analysis of randomised controlled trials (RCTs) indeed showed a reduced risk of fracture with use of DPP4-Is[7]. Recently, we have performed the first observational studies investigating the association between use of DPP4-Is and risk of fracture[8,9]. In contrast to the result of the meta-analysis, we found no association between use of DPP4-Is and fracture risk. One of the explanations might be the fact that the meta-analysis was based on a small number of fractures, which needed to be reported as severe adverse outcomes. In contrast, the observational studies used routinely collected data on fractures. Recently, another observational study was published which compared current use of metformin and DPP4-Is to non-use. Non-use was defined as insufficient exposure to diabetes medication (i.e., a medication possession ratio (MPR) <20%)[10]. This study showed no association with fracture risk when a DPP4-I was added to metformin.

A major limitation of both the observational studies as well as the meta-analysis was the median actual duration of DPP4-I use. For the observational studies it ranged between 47 weeks[9] and 1.04 years[8] and for the meta-analysis the median duration of the included trials was 24 weeks[7].

Based on these findings, the limited duration of DPP4-I use might have been too short to show an association between use of DPP4-Is and fracture risk. Therefore, in the present study we aimed to investigate the association between long-term use of DPP4-Is and risk of fracture.

Methods

Data for this study were obtained from the Clinical Practice Research Datalink (CPRD) in the United Kingdom, previously known as the General Practice Research Database (GPRD) [www.CPRD.com]. The CPRD contains computerized medical records of 674 primary care practices in the United Kingdom, representing 6.9% of the population[11]. The data recorded in the CPRD include demographic information, prescription details, clinical events, preventive care provided, specialist referrals, hospital admissions, and major outcomes since 1987. Previous studies using CPRD data have shown to be highly valid, with for example for hip fractures over 90% confirmed diagnoses[12].

We conducted a retrospective population-based cohort study. The study population consisted of all patients with at least one prescription for a non-insulin anti-diabetic drug (NIAD) and who were aged 18+ during the period of valid CPRD data collection. For this study, data collection started on June 13, 2007, the date of the first ever prescription of a DPP4-I in CPRD, and ended December 31, 2015. The index date was defined as the date of the first NIAD prescription since the start of the study period (i.e. the study population was a mix of incident and prevalent NIAD users). This study obtained approval from the independent scientific advisory committee of the CPRD (protocol number: 12_161R).

Exposure

The follow-up time of the NIAD users was divided into fixed intervals of 30 days. When there was a prescription of a NIAD in the 90 days before the start of an interval, the interval was classified as current NIAD use, otherwise the interval was classified as past NIAD use. Patients were allowed to move between current and past NIAD use. All DPP4-I exposed intervals were classified, according to the time since the most recent prescription, as current (1-90 days), recent (91-180 days), or past (over 180 days) use.

Continuous duration of use was determined at the start of every interval. The prescribed quantity and the written dosage instruction were used to estimate the duration of each DPP4-I prescription. Continuous duration of use was defined as the time from the first continuous prescription until the start of an interval, allowing a gap of 30 days[13] between the estimated end date of a prescription and the start of the next prescription.

Outcome

Patients were followed up from the index date to either the end of data collection, the date of transfer of the patient out of the practice area, the patient's death, or the fracture type of interest, whichever came first. Fractures were classified by use of read codes[14]. We used the following categories to classify fractures: any, hip, and osteoporotic fracture. An osteoporotic fracture was defined as a fracture of the hip, vertebrae, radius/ulna or humerus according to the WHO definition[15].

Potential confounders

The presence of risk factors was assessed by reviewing the computerized medical records for any record of a risk factor prior to the start of an interval. The following potential confounders were determined at baseline: sex, body mass index (BMI), smoking status and alcohol use. All other risk factors that were considered in this study were determined time-dependently (i.e. at the start of each interval). We considered the following potential confounders: age, most recent glycosylated hemoglobin A1c

(HbA1c) measurement in the year prior the start of an interval, falls in 7-12 months before the start of an interval, a history of chronic obstructive pulmonary disease, previous fracture, rheumatoid arthritis, hypothyroidism, hyperthyroidism, cancer, retinopathy, neuropathy, congestive heart failure and secondary osteoporosis (hypogonadism or premature menopause). In addition, the following drug prescriptions in the 6 months prior to the start of an interval were considered as a potential confounder: oral glucocorticoids, cholesterol modifying drugs, antidepressants, anxiolytics or hypnotics, antipsychotics, anti-Parkinson drugs, antihypertensives (beta-blockers, thiazide diuretics, renin angiotensin aldosteron system inhibitors, calcium channel blockers, loop diuretics), antiarrhythmics, opposed hormone replacement therapy, calcium, bisphosphonates, vitamin D, raloxifene, strontium ranelate, calcitonin and parathyroid hormone.

Statistical analyses

Regression analysis with Cox proportional hazards models (SAS 9.4, PHREG procedure) was used to estimate the fracture rate of current DPP4-I users compared to other NIAD users, excluding glucagon-like peptide 1 receptor agonist (GLP1-RA) users. GLP1-RA use was taken into account as a separate exposure group as it has been associated with a decreased risk of fracture[16]. In further analyses we stratified current DPP4-I use by categories of continuous duration of use. In all analyses potential confounders were included if they independently changed the beta-coefficient for current DPP4-I exposure by at least 5%, or when consensus about inclusion existed within the team of researchers, supported by clinical evidence from literature. For confounder data with missing values (BMI, HbA1c, alcohol use and smoking status) a missing indicator variable was added.

Sensitivity analyses

As a sensitivity analysis the gap used to determine continuous duration of use was changed to 60 and 90 days. In a second sensitivity analysis we performed a new-user design in which all NIAD users with a NIAD prescription before the start of the study were excluded from the analyses. Additionally, we performed a sensitivity analysis in which all patients with a history of a fracture before the index date were excluded. A fourth sensitivity analysis was performed in which we adjusted the main analyses for current use of thiazolidinediones and current use of sulfonylurea derivatives, as they have been associated with fracture risk, respectively[17,18].

Results

In total 328,254 NIAD users were included, of which 46,355 were DPP4-I users. The baseline characteristics are shown in Table 2.7.1. The median actual duration of DPP4-I use was 1.6 years and the mean duration of follow-up was 6.3 and 5.6 years for the DPP4-I users and the NIAD users, respectively. DPP4-I users were less often women and slightly younger, had a higher HbA1c and BMI at index date as compared to other NIAD users (HbA1c: 8.8 versus 8.0 and BMI: 32.6 versus 31.4). History of retinopathy, use of statins and use of antihypertensives was higher in DPP4-I users at baseline as compared to other NIAD users.

Table 2.7.1 Baseline characteristics of current DPP4-I users and other NIAD users.

Characteristic	DPP4-I users N=46,355	Other NIAD users N=281,899
Mean follow-up time (years, SD)	6.3 (2.5)	5.6 (2.8)
Median actual duration of DPP4-use, [IQR]	1.6 [0.7 – 3.1]	n/a
Females	19,428 (41.9)	114,467 (48.6)
Age		
Mean age at index date (years, SD)	59.7 (12.4)	61.5 (16.1)
18 - 49 years	9,883 (21.3)	53,131 (22.6)
50 - 59 years	12,550 (27.1)	44,660 (19.0)
60 - 69 years	13,448 (29.0)	56,812 (24.1)
70 - 79 years	8,065 (17.4)	50,561 (21.5)
80+ years	2,409 (5.2)	30,380 (12.9)
BMI		
Mean BMI at index date (kg/m^2, SD)	32.6 (6.7)	31.4 (6.8)
<20.0 kg/m^2	273 (0.6)	3,646 (1.5)
20.0 - 24.9 kg/m^2	4,044 (8.7)	30,956 (13.1)
25.0 - 29.9 kg/m^2	13,270 (28.6)	71,599 (30.4)
30.0 - 34.9 kg/m^2	14,059 (30.3)	63,347 (26.9)
≥35.0 kg/m^2	14,023 (30.3)	57,971 (24.6)
Missing	686 (1.5)	8,025 (3.4)
HbA1c		
Mean HbA1c (%, SD)	8.8 (1.5)	8 (1.8)
<6%	394 (0.8)	8,550 (3.6)
6.0 - 6.9%	2,807 (6.1)	35,868 (15.2)
7.0 - 7.9%	10,418 (22.5)	42,017 (17.8)
8.0 - 8.9%	12,217 (26.4)	22,598 (9.6)
≥9.0%	15,720 (33.9)	31,355 (13.3)
Missing	4,799 (10.4)	95,156 (40.4)
Smoking status		
Never	14,839 (32.0)	77,335 (32.8)
Current	8,015 (17.3)	40,256 (17.1)
Ex	23,334 (50.3)	116,180 (49.3)
Missing	167 (0.4)	1,773 (0.8)
Alcohol use		
No	14,349 (31.0)	74,826 (31.8)
Yes	30,416 (65.6)	145,609 (61.8)
Missing	1,590 (3.4)	15,109 (6.4)
Falls (6 - 12 months before index date)	477 (1.0)	2,427 (1.0)

Table 2.7.1 (continued)

Characteristic	DPP4-I users N=46,355	Other NIAD users N=281,899
History of diseases		
Fracture	9,575 (20.7)	49,438 (21.0)
Hyperthyroidism	535 (1.2)	2,315 (1.0)
Hypothyroidism	4,180 (9.0)	18,997 (8.1)
COPD	2,765 (6.0)	12,559 (5.3)
Congestive heart failure	1,929 (4.2)	9,119 (3.9)
Cancer	11,491 (24.8)	51,587 (21.9)
Rheumatoid arthritis	726 (1.6)	3,621 (1.5)
Retinopathy	12,976 (28.0)	31,532 (13.4)
Secondary osteoporosis	3,774 (8.1)	19,655 (8.3)
Neuropathy	3,090 (6.7)	10,506 (4.5)
Drug use within six months prior to index date		
Glucocorticoids	9,160 (19.8)	40,683 (17.3)
Statins	34,574 (74.6)	118,488 (50.3)
Antiarrhythmics	641 (1.4)	3,464 (1.5)
Antidepressants	9,688 (20.9)	38,909 (16.5)
Anti-Parkinson drugs	259 (0.6)	1,235 (0.5)
Antipsychotics	997 (2.2)	5,794 (2.5)
Anxiolytics/hypnotics	3,133 (6.8)	16,402 (7.0)
Antihypertensives	32,411 (69.9)	129,584 (55.0)
Bisphosphonates	1,032 (2.2)	6,399 (2.7)
Raloxifene	14 (0.0)	282 (0.1)
Calcium/vitamin D	2,393 (5.2)	10,574 (4.5)
Strontium	16 (0.0)	126 (0.1)
Parathyroid hormone/calcitonin	<6 (0.0)	<6 (0.0)
Hormone replacement therapy	178 (0.4)	820 (0.3)

Data are presented as number (%), unless stated otherwise. BMI: body mass index; DPP4-I: dipeptidyl peptidase 4 inhibitor; HbA1c: glycosylated hemoglobin A1c; IQR: inter quartile range; n/a: not applicable; NIAD: non-insulin anti-diabetic drug; SD: standard deviation

Table 2.7.2 shows that any DPP4-I use was associated with a decreased risk of any fracture (adjusted (adj.) hazard ratio (HR): 0.93 (95% confidence interval (CI) 0.88-0.94)). Current use of DPP4-Is was not associated with risk of any fracture (adj. HR: 0.99 (95% CI: 0.93-1.06)) as compared to current other NIAD use. Recent DPP4-I use was not associated with risk of fracture either, whereas, past DPP4-I use was associated with a decreased risk of fracture (adj. HR: 0.83 (95% CI: 0.75-0.91)). NIAD past use was associated with a 60% reduced risk of any fracture (adj. HR: 0.40 (95% CI: 0.38-0.43)). The fully adjusted model including all potential confounders showed an HR of 0.95 (95% CI: 0.89-1.01) with current use of DPP4-I and risk of any fracture. Stratification by continuous duration of use resulted in an increased risk of fracture for patients who continuously used DPP4-I for 2.0-2.9 years (adj. HR: 1.23 (95% CI: 1.03-1.48)). Other categories showed no association with continuous duration of DPP4-I use, all Table 2.7.2.

Table 2.7.2 Use of DPP4-Is and risk of any fracture stratified by continuous duration of use.

	Number of fractures N=16,572[a]	IR / 1000 PY	Age/gender adjusted HR (95% CI)	Adjusted HR (95% CI)[b]
Current NIAD excl. incretins	12,575	14.3	Reference	Reference
Past NIAD	1,923	3.3	0.23 (0.22 - 0.24)*	0.40 (0.38 - 0.43)*
Any DPP4-I	1,700	10.3	0.88 (0.83 - 0.92)*	0.93 (0.88 - 0.94)*
By recency				
Past DPP4-I	479	7.6	0.65 (0.59 - 0.72)*	0.83 (0.75 - 0.91)*
Recent DPP4-I	93	9.9	0.84 (0.68 - 1.03)	0.83 (0.67 - 1.02)
Current DPP4-I	1,128	12.2	1.02 (0.96 - 1.08)	0.99 (0.93 - 1.06)
By continuous duration of use				
No continuous duration of use	275	12.0	1.00 (0.89 - 1.13)	0.97 (0.86 - 1.09)
≤0.5 year	311	12.2	1.01 (0.90 - 1.13)	1.00 (0.89 - 1.12)
0.6 - 0.9 year	162	11.6	0.96 (0.82 - 1.12)	0.93 (0.80 - 1.09)
1.0 - 1.9 years	192	12.4	1.02 (0.88 - 1.18)	1.00 (0.87 - 1.16)
2.0 - 2.9 years	117	14.9	1.25 (1.04 - 1.50)*,[c]	1.23 (1.03 - 1.48)*,[c]
3.0 - 3.9 years	40	9.9	0.86 (0.63 - 1.17)	0.84 (0.62 - 1.15)
4.0 - 8.5 years	31	11.0	1.02 (0.72 - 1.45)	0.99 (0.70 - 1.41)

DPP4-I: dipeptidyl peptidase 4 inhibitor; GLP1-RA: glucagon-like peptide 1 receptor agonist; HbA1c: glycosylated hemoglobin A1c; HR: hazard ratio; IR: incidence rate; NIAD: non-insulin anti-diabetic drug; py: person years. Current NIAD use: most recent NIAD prescription within 90 days before start of an interval. Past NIAD use: most recent prescription over 90 days before start of an interval. Past DPP4-I use: most recent prescription over 180 days before start of an interval. Recent DPP4-I use: most recent prescription within 91-180 before start of an interval. Current DPP4-I use: most recent prescription within 90 before start of an interval. * Statistically significant (p-value <0.05). [a] GLP1-RA use not shown, therefore the total numbers of fractures do not add up to the total number of fractures. [b] Adjusted for: age, gender, body mass index, smoking status, HbA1c, use of antipsychotics, glucocorticoids, statins, anti-depressants, anti-hypertensives, anxiolytics/hypnotics, calcium/vitamin d, anti-osteoporotic drugs[d], history of fracture, falls, secondary osteoporosis, retinopathy and neuropathy. [c] Statistically significant difference compared with no continuous duration of use, 0.5-1 year of continuous duration of use and 3-4 years of continuous duration of use, using Wald-test (p<0.05). [d] Use of bisphosphonates, raloxifene, strontium ranelate or parathyroid hormone/ calcitonin.

Any DPP4-I use was associated with a decreased risk of osteoporotic fracture but not with hip fracture (adj. HR osteoporotic fracture: 0.91 (95% CI: 0.84-0.98); hip fracture: 0.92 (95% CI: 0.79-1.06)). Current use of DPP4-Is was not associated with risk of osteoporotic (adj. HR: 0.96 (95% CI: 0.87-1.05)) or hip fracture (adj. HR: 0.96 (95% CI: 0.81-1.15)), Table 2.7.3. Both recent and past DPP4-I use showed a decreased risk of osteoporotic fracture (adj. HR recent DPP4-I use: 0.72 (95% CI: 0.52-0.99), past DPP4-I use: 0.84 (95% CI: 0.73-0.96)). Recent and past DPP4-I use were not associated with risk of hip fracture. Past NIAD use was associated with a reduced risk of major osteoporotic fracture and hip fracture. Current use of DPP4-Is stratified by continuous duration of use was not associated with risk of osteoporotic fracture nor with risk of hip fracture.

Table 2.7.3 Use of DPP4-Is and risk of osteoporotic and hip fracture, stratified by continuous duration of use.

	Number of fractures N=8,046[a]	IR / 1000 PY	Age/gender adjusted HR (95% CI)	Adjusted HR (95% CI)[b]		Number of fractures N=2,737[a]	IR / 1000 PY	Age/gender adjusted HR (95% CI)	Adjusted HR (95% CI)[b]
Osteoporotic fracture					Hip fracture				
Current NIAD excl. incretins	6,952	6.9	Reference	Reference	Current NIAD excl. incretins	392	2.3	Reference	Reference
Past NIAD	952	1.6	0.20 (0.19 - 0.22)*	0.37 (0.34 - 0.41)*	Past NIAD	2,110	0.6	0.19 (0.17 - 0.22)*	0.38 (0.34 - 0.44)*
Any DPP4-I	755	4.4	0.83 (0.77 - 0.90)*	0.91 (0.84 - 0.98)*	Any DPP4-I	223	1.3	0.82 (0.71 - 0.94)*	0.92 (0.79 - 1.06)
By recency					By recency				
Past DPP4-I	220	3.4	0.64 (0.55 - 0.73)*	0.84 (0.73 - 0.96)*	Past DPP4-I	68	1.0	0.64 (0.50 - 0.83)*	0.91 (0.71 - 1.17)
Recent DPP4-I	37	3.8	0.72 (0.52 - 1.00)*	0.72 (0.52 - 0.99)*	Recent DPP4-I	8	0.8	0.52 (0.25 - 1.05)	0.51 (0.25 - 1.02)
Current DPP4-I	498	5.2	0.97 (0.89 - 1.07)	0.96 (0.87 - 1.05)	Current DPP4-I	147	1.5	0.96 (0.81 - 1.14)	0.96 (0.81 - 1.15)
By continuous duration of use					By continuous duration of use				
No continuous duration of use	113	4.8	0.88 (0.73 - 1.06)	0.86 (0.71 - 1.04)[c]	No continuous duration of use	37	1.5	0.95 (0.68 - 1.31)	0.94 (0.68 - 1.30)
≤0.5 year	155	5.9	1.11 (0.95 - 1.31)	1.11 (0.94 - 1.30)	≤0.5 year	40	1.5	0.98 (0.72 - 1.35)	1.00 (0.73 - 1.37)
0.6 - 0.9 year	67	4.7	0.86 (0.68 - 1.10)	0.85 (0.67 - 1.08)	0.6 - 0.9 year	19	1.3	0.83 (0.52 - 1.30)	0.82 (0.52 - 1.29)
1.0 - 1.9 years	80	5.0	0.91 (0.73 - 1.14)	0.90 (0.72 - 1.13)	1.0 - 1.9 years	22	1.4	0.84 (0.55 - 1.29)	0.85 (0.56 - 1.29)
2.0 - 2.9 years	55	6.7	1.26 (0.96 - 1.64)[d]	1.24 (0.95 - 1.62)[d]	2.0 - 8.5 years	29	1.9	1.23 (0.85 - 1.77)	1.24 (0.85 - 1.79)
3.0 - 8.5 years	28	3.9	0.77 (0.53 - 1.12)	0.75 (0.52 - 1.09)					

DPP4-I: dipeptidyl peptidase 4 inhibitor; GLP1-RA: glucagon-like peptide 1 receptor agonist; HR: hazard ratio; IR: incidence rate; NIAD: non-insulin anti-diabetic drug; py: person years. Current NIAD use: most recent NIAD prescription within 90 days before start of an interval. Past NIAD use: most recent prescription over 90 days before start of an interval. Past DPP4-I use: most recent prescription over 180 days before start of an interval. Recent DPP4-I use: most recent prescription within 91-180 before start of an interval. Current DPP4-I use: most recent prescription within 90 before start of an interval. * Statistically significant (p-value <0.05). [a] GLP1-RA use not shown, therefore the total numbers of fractures do not add up to the total number of fractures. [b] Adjusted for: age, gender, body mass index, smoking status, glycosylated hemoglobin A1c, use of antipsychotics, glucocorticoids, statins, anti-depressants, anti-hypertensives, history of congestive heart failure, fracture, falls, secondary osteoporosis, retinopathy and neuropathy. [c] Statistically significant difference compared with ≤0.5 year of continuous duration of use, using Wald-test (p<0.05) [d] Statistically significant difference compared with no continuous duration of use, 0.5-1 year of continuous duration of use and 3-8.5 years of continuous duration of use, using Wald-test (p<0.05).

In the first sensitivity analysis we extended the gap between the expected end date of a prescription and the start of the next prescription to 60 and 90 days, respectively. When the gap was set to 60 days the results for risk of any, osteoporotic or hip fracture did not substantially change except for continuous duration of DPP4-I use of 2.0-2.9 years and risk of any fracture, which was not significant anymore (adj. HR: 1.04 (95% CI: 0.87-1.24)). When the gap was set to 90 days similar results were seen: no materially altered results for risk of any, osteoporotic or hip fracture except with 2.0-2.9 years of DPP4-I use and risk of any fracture, which was not significantly any more (adj. HR: 1.04 (95% CI: 0.88-1.24)).

In a second sensitivity analysis the study population was restricted to new users of NIADs. Current DPP4-I use stratified by continuous duration of use categories was not associated with risk of any fracture. Additional adjustment for diabetes duration did not materially alter the results. In a third sensitivity analysis we excluded all patients with a history of a fracture. Current use of DPP4-Is stratified by continuous duration of use was not associated with risk of any fracture. Additional adjustments for current use of thiazolidinediones and sulfonylurea derivatives resulted in a significantly decreased risk of any fracture with current DPP4-I (adj. HR: 0.94 (95% CI: 0.88-1.00)).

Discussion

The present study showed that current use of DPP4-Is was not associated with risk of any, osteoporotic or hip fracture. After stratification by continuous duration of use we showed no association between the highest continuous duration of use category with any (>4.0-8.5 years of DPP4-I use), osteoporotic (>3.0-8.5 years of use) or hip (>2.0-8.5 years of use) fracture. Different sensitivity analyses confirmed the results of no association between current use of DPP4-Is and risk of any fracture.

Our results are in line with the results of two recently performed meta-analyses, which included 51 and 62 RCTs, respectively, comparing DPP4-Is to placebo or an active comparator[19,20]. Both meta-analyses showed no association between use of DPP4-I and risk of fracture. The adverse events fracture data of a large clinical trial (n=16,492) comparing saxagliptin, a DPP4-I, to placebo have been analysed in more depth and showed a relative risk of 1[21]. The present results are also supported by the results of an analysis on the fracture data of a cardiovascular trial comparing sitagliptin, a DPP4-I, to placebo in patients with T2DM (n=14,671) which showed no association with risk of fracture[22].

The present results are also in keeping with our previous observational studies comparing current use of DPP4-Is to other NIADs[8,9], with a meta-analysis of the observational studies[23] and with an observational study comparing use of metformin and DPP4-I to non-use[10]. The present results are not in line with a meta-analysis including 28 RCTs comparing DPP4-Is to placebo or active treatment which showed a

40% reduced risk[7]. However, the two updated meta-analyses showed no reduced risk of fracture with use of DPP4-I[19,20], suggesting that the large reduction of fracture risk found in the first meta-analysis might have been a consequence of the small number of included trials and the small number of reported fractures.

DPP4-Is increase the concentration of incretin hormones glucagon-like peptide 1 (GLP1) and gastric inhibitory polypeptide (GIP)[5]. In vitro research has shown that GIP stimulates osteoblast differentiation[24]. Treatment with GLP1 has been associated with an increase in bone density in rodent models with osteopenia[25,26]. It was therefore hypothesized that DPP4-Is may reduce fracture risk. However in the current study we did not show a decreased risk of fracture with use of DPP4-Is. One of the explanations might be that DPP4-Is are still not used long enough to establish this reduced risk of fracture. However, a small group of patients used DPP4-Is continuously for more than four years, when studying risk of any fracture. Anti-hyperglycaemic drugs that have been associated with an unintended effect on bone, such as thiazolidinediones, showed this already after two years of use[27,28]. In addition, bisphosphonates, used to prevent fractures, have shown a reduction in fracture risk after 18 months of use[29,30]. GIP and GLP1 have shown to be reduced in patients with T2DM[31]. It might be that due to the use of DPP4-Is the levels of GIP and GLP1 increase to the normal level, but not to higher levels required to have an effect on bone metabolism and in the end on fracture risk.

Current use of DPP4-Is was associated with an increased risk of any fracture when patients used it continuously for 2.0-2.9 years. Longer use was not associated with an increased fracture risk, which would be expected if DPP4-I use would increase fracture risk. In addition, in all sensitivity analyses this increased risk disappeared, suggesting that this increased risk was a chance finding. Unexpectedly, past NIAD use was associated with a 60% reduced risk of any fracture, which is hard to explain and should be interpreted with caution. Past NIAD use includes patients who are switched to insulin use. However, this has been associated with an increased and not a decreased risk of fracture[32].

Strengths of this study include its large sample size as well as the representativeness of the used CPRD data for the general population of the United Kingdom. In addition, we were able to adjust for many potential important confounders in a time-dependent manner. We also had data on important life-style factors such as BMI and we had data on HbA1c. Moreover, we were able to investigate the association between current use of DPP4-Is for >4.0-8.5 years and risk of any fracture. Additionally, it has been shown that the CPRD fracture data has a high validity[12].

Our study had also some limitations. For osteoporotic and hip fracture we had to lump the highest categories of continuous duration of use into >3.0-8.5 years and >2.0-8.5 years, respectively. Another limitation is that despite the fact that the follow-up period was extended with almost 3.5 years as compared to the previous observational studies[8,9], the median duration of actual DPP4-I use only increased with 0.6 year. Future work could be beneficial to properly evaluate the associated between

long duration of DPP4-I use and the effects on fracture risk, once the duration data has had sufficient time to mature. Moreover, although we were able to adjust for many confounders, residual confounding may be present.

We showed that current use of DPP4-Is was not associated with risk of any, osteoporotic, or hip fracture. Moreover, we showed that, when stratified by continuous duration of use, current use of DPP4-Is was not associated with a decreased risk of any (>4.0-8.5 years of DPP4-I use), osteoporotic (>3.0-8.5 years of DPP4-I use), or hip (>2.0-8.5 years of DPP4-I use) fracture. These findings may be of value for clinical decisions regarding treatment of T2DM patients, especially those at high fracture risk.

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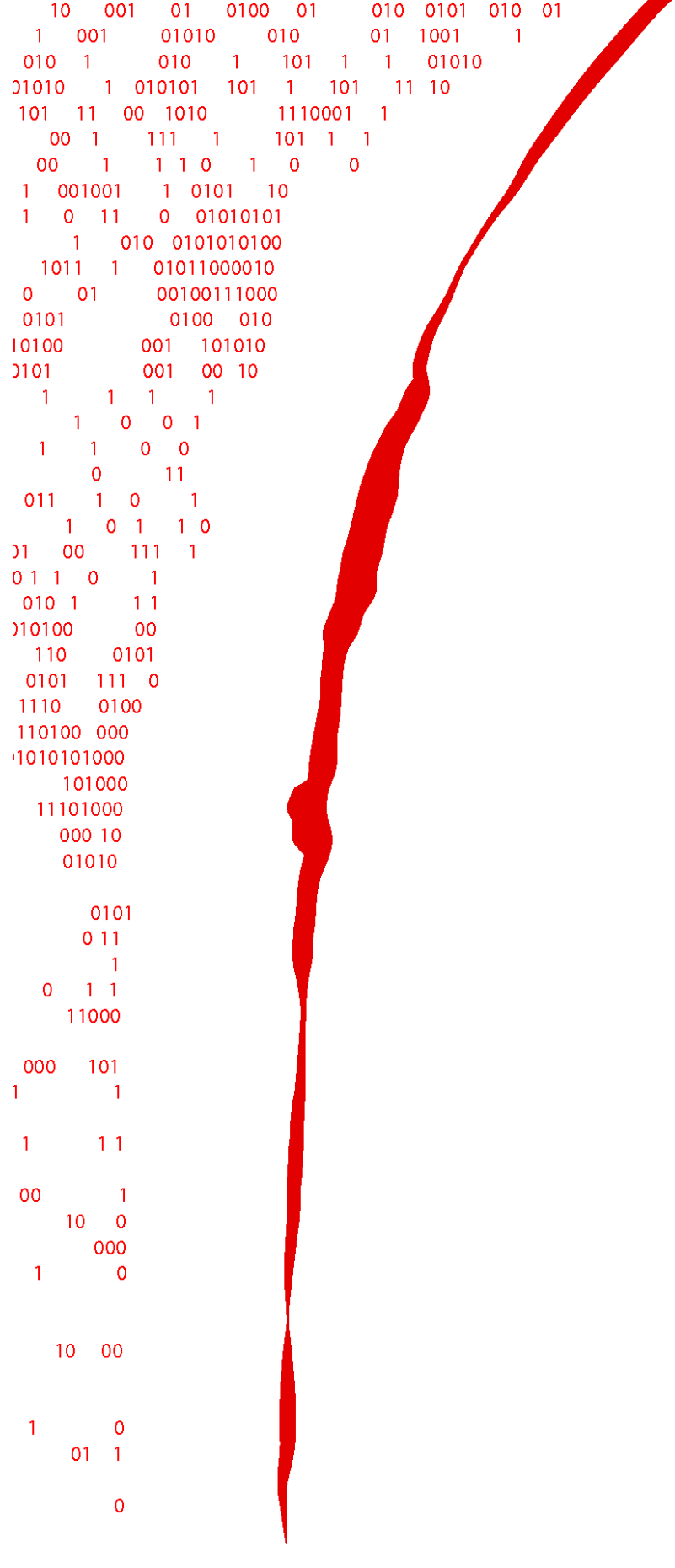

Chapter 3

Anti-hyperglycaemic drug use within the Maastricht Study population and potential unintended effects

Chapter 3.1

Drug utilisation in the Maastricht Study: a comparison with nationwide data

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Submitted

Chapter 3.2

The association between insulin use and volumetric bone mineral density, bone micro-architecture and bone strength of the distal radius in patients with T2DM – the Maastricht Study

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Pending Revisions

Chapter 3.3

Metformin dosage and duration is not associated with aortic stiffness – the Maastricht Study

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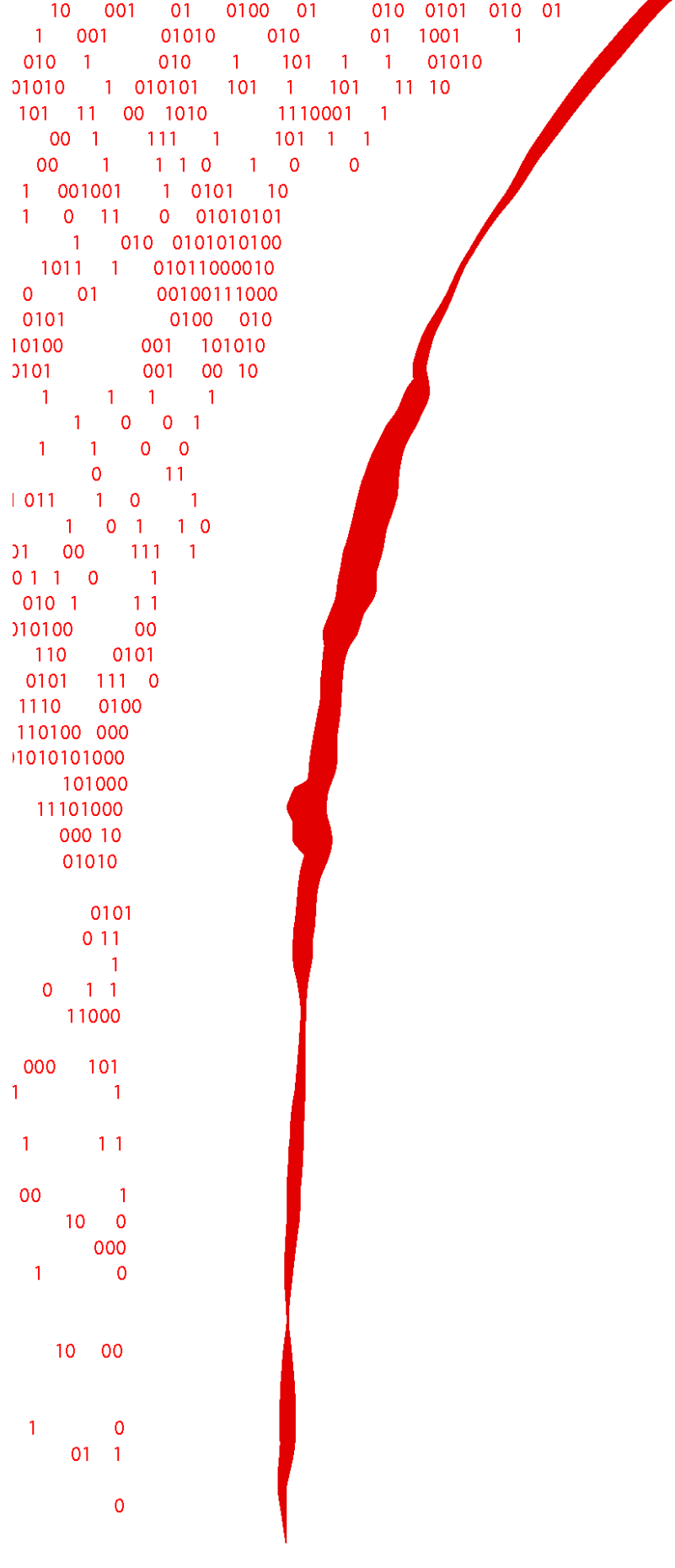

Chapter 4

Methodological Aspects

Chapter 4.1

Evaluation of different missing data strategies in propensity score analyses

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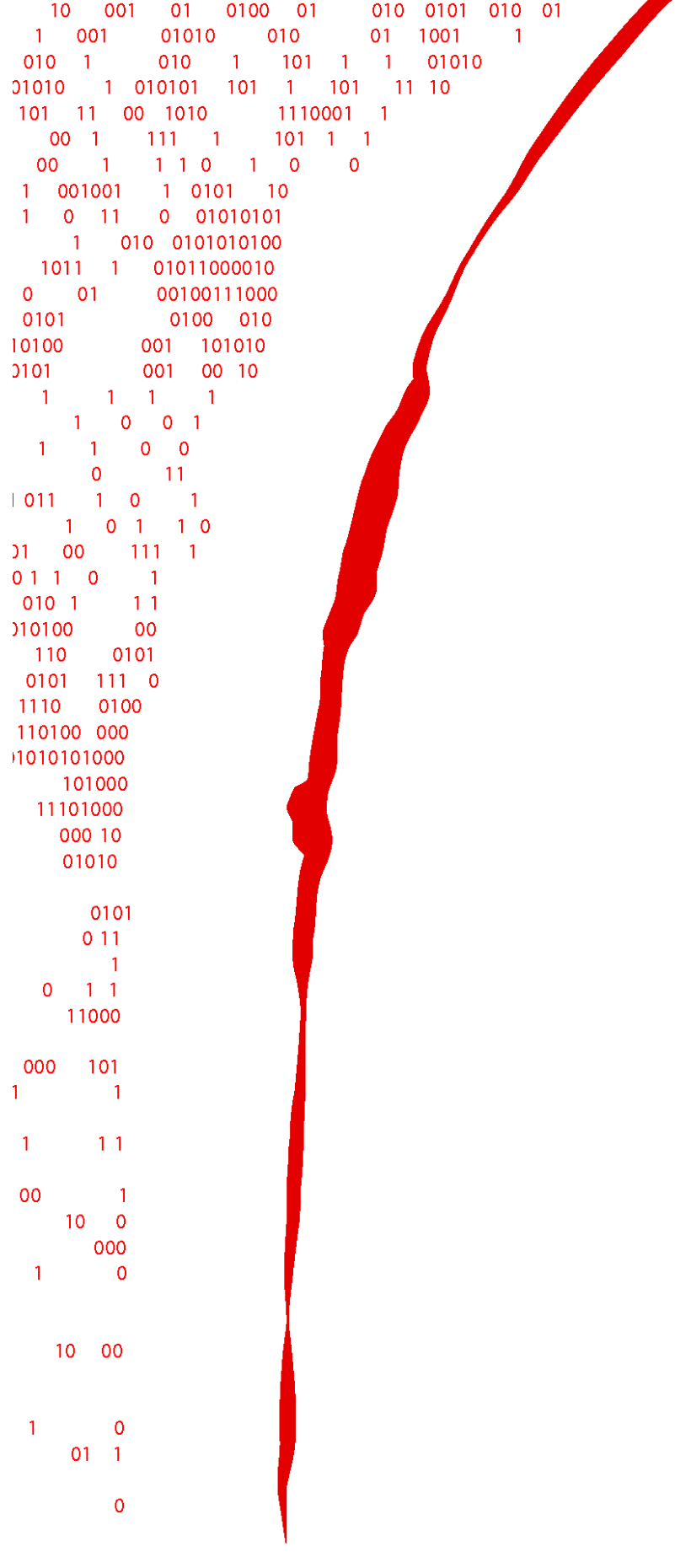

Chapter 5

General Discussion

This thesis evaluated several unintended effects of various classes of anti-hyperglycaemic drugs in population-based cohorts. In Chapter 2 we evaluated the association between use of incretin agents, both dipeptidyl peptidase 4 inhibitors (DPP4-Is) and glucagon-like peptide 1 receptor agonists (GLP1-RAs), and risk of fracture. Thereafter we assessed whether data from the Maastricht Study could be used to evaluate unintended effects of diabetes medication (Chapter 3). Finally, we studied the robustness of an estimated association when using a range of approaches to handle missing data within a propensity score analyses. In this final chapter the main findings and the clinical implications will be discussed. This is then followed by a discussion of methodological considerations and future perspectives. This thesis will conclude with clinical and methodological recommendations based on the results of this thesis.

Main findings

The aim of Chapter 2 was to investigate the association between the use of incretin agents and fracture risk. We did not find an association between short-term use of DPP4-Is or GLP1-RAs and risk of fracture using data representative for the United Kingdom (UK) as well as data representative for Denmark. Additionally, we did not find an association between use of these incretin agents after meta-analysing our results. Moreover, long-term use (>4 years) of DPP4-Is was not associated with risk of fracture either. These findings are in line with the results from a large clinical trial (n=16,492), demonstrating no association with fracture risk when comparing DPP4-I use to placebo[1,2]. Two recent meta-analyses of 51 and 62 randomised controlled trials (RCTs), respectively, comparing DPP4-Is to an active comparator or placebo also showed no association[3,4]. Data from RCTs comparing GLP1-RAs to active comparator or placebo have also been meta-analysed. No overall effect has been found[5,6]. However, when GLP1-RAs were stratified by type, a reduced risk of fracture was reported with liraglutide while users of exenatide had an elevated risk[6].

Based on the data presented in this thesis and together with data from literature it can be concluded that there is no beneficial effect of incretin agents on fracture risk. Hence, these findings may be of value for clinical decisions regarding treatment of patients with T2DM, especially those at high fracture risk.

The aim of Chapter 3 was to assess the usefulness of the Maastricht Study data to evaluate unintended effects of diabetes medication (Chapter 3). After evaluating the representativeness of the data we first explored the association between use of insulin as compared to non-insulin use and bone micro-architecture and strength parameters. Secondly, we studied the association between use of metformin and carotid-femoral pulse wave velocity (cfPWV), as a measure for arterial stiffness.

Participants visit the Maastricht Study four to five times for the extensive assessment of various measurements[7]. This might have resulted in a selected

population as only patients who were able and willing to undergo this extensive procedure were included. The first published studies showed indeed that participants of the Maastricht Study were on average younger, had a higher level of education and were healthier than the source population[8-10]. However, it was unknown whether this was also present with respect to the dispensing data of the Maastricht Study participants. Therefore we compared the dispensing data of the Maastricht Study to drug reimbursement data of the total Dutch population.

For most of the investigated drugs we found similar results between the national data and the Maastricht Study data, except for respiratory prescriptions and hypnotics/anxiolytics. One of the hypotheses for this decreased number of respiratory prescriptions is the fact that these drugs are often prescribed for asthma and/or chronic obstructive pulmonary disease (COPD). Guidelines recommend that patients with severe asthma or COPD needs to be followed-up frequently[11,12]. Patients with severe asthma or COPD might therefore be less willing or able to participate in the Maastricht Study. This then could partially explain the lower number of respiratory prescriptions.

The use of hypnotics/anxiolytics was significantly higher in the study population, as compared to the national population. One of the hypothesis is that the participants of the Maastricht Study are in general healthier but also more care seeking as compared to the Dutch population[13].

Anti-hyperglycaemic drug use was comparable between the national data and the Maastricht Study data. However, a report on national prescription data showed that the average number of citizens who had at least one drug prescription was higher in the South-Limburg region as compared to national average in 2013 (720 vs. 677 per 1000 insured citizens, respectively)[14]. This was not reflected in the Maastricht Study dispensing data, which is in agreement with earlier results suggesting that participants of the Maastricht Study are relatively healthy[8-10]. An advantage of the selected Maastricht Study population is the fact that it enables the study of potential unintended effects in patients with a relatively short duration of T2DM and with only a few comorbidities.

As a consequence of the relatively healthy Maastricht Study population the studied associations may be underestimated as patients with more severe T2DM are less likely to be present in the Maastricht Study. However, it can also be hypothesized that the associations are overestimated. A case-control study investigating the association between stroke and risk of fracture showed that risk of fracture was greatest in younger patients[15]. It was hypothesized that older patients have multiple risk factors increasing fracture risk, whereas this is less the case in younger patients resulting in a relatively larger contribution of stroke to fracture risk in the younger patients.

Then two different pharmacoepidemiological studies using data from the Maastricht Study were performed. Chapter 3.2 was a small explorative study investigating the potential association between use of insulin compared to non-insulin

use and bone micro-architecture and strength parameters. A larger number of patients is needed before we can draw definite conclusions about the potential association. Nevertheless, the explorative results showed that use of insulin was negatively associated with bone micro-architectural and bone strength parameters.

Based on the results from Chapter 3.3 we concluded that metformin dosage and duration was not associated with a beneficial effect on arterial stiffness, as measured by cfPWV. However, the relatively healthy population may be an alternative explanation for the fact that we did not find an association. Additionally, we concluded that, although investigated in a small population, metformin was not associated with plasma advanced glycation end products (AGEs) or AGEs measured by skin autofluorescence. This is in line with the results of a RCT, which investigated whether metformin may have beneficial effects on AGEs[16]. This led to the hypothesis that not metformin itself but its anti-hyperglycaemic effect reduces the level of plasma AGEs.

The studies presented in Chapter 3.2 and 3.3 showed that it is possible to perform pharmacoepidemiological research using data from the Maastricht Study. Examples of the extensive phenotyping in the Maastricht Study include the measurement of the bone architectural and bone strength parameters as well as the measurement of arterial stiffness via cfPWV. These measurements are generally not present in electronic healthcare databases (EHDs). Moreover there is longitudinal dispensing data available in the Maastricht Study which makes it an unique database to perform pharmacoepidemiological research. The results from Chapter 3.2 and 3.3 cannot be translated into clinical implications yet, as larger number of patients and longitudinal data is needed before definite conclusion can be drawn.

The last aim of this thesis was to investigate the effect on treatment estimates when different methods to handle missing data are combined with propensity score analysis. From the case study in Chapter 4.1 it can be concluded that combining propensity scores with different methods to handle missing data may result in similar treatment estimates as compared to multivariable adjusted estimated when performing time-fixed time-to-event analyses. Further, more research is needed whether this also applies to time-dependent time-to-event analyses.

Methodological considerations of the work presented in this thesis

Although the studies presented in this thesis had different strengths, including the use of population-based data and large sample sizes, the results also need to be viewed in the light of some methodological considerations. When interpreting the results of the studies presented in this thesis some potential sources of bias need to be taken into account.

Bias is defined as a systematic error in the design of the study or as a systematic error in the collection, analysis, interpretation, reporting, or review of the data, which leads to results which are systematically different from the truth[17]. Most biases can be classified as either information bias, selection bias or confounding. Information bias occurs when there is incorrect information on exposure, outcome or confounding variables. Selection bias happens when study participants are not representative for the outcome or exposure in the source population due to selective recruitment. Confounding is the result of an imbalance of covariates between the different compared groups which results in a biased estimate of the effect measure[18].

Bias might seriously distort the estimated effect measures and should therefore be avoided as much as possible. It is not possible to correct analyses for bias. The only way to minimize bias is by a proper study design[19]. The results of the studies presented in this thesis will now be considered in light of some potential sources of bias.

Information bias

Misclassification bias

Data on dispensed prescriptions is often used to determine drug exposure in pharmacoepidemiological studies. However, this does not necessary reflect whether patients actually took the drug or not. Therefore, this may result in misclassification bias as patients are classified as exposed while they actually were not exposed[18]. When this occurs equally between the studied groups, or when the misclassification is independent of the outcome or exposure it is said to be non-differential[18]. If the exposure is dichotomous this non-differential misclassification bias results in an effect towards the null. However, when there are more than two exposure categories the direction of the bias can be either towards or away from the null, depending on the exposure categories in which the misclassification occurs[20-22]. Non-differential misclassification bias might have occurred in all studies (except Chapter 2.1) in this thesis. It is hard to determine the direction of the bias as there were often more than two exposure categories in the presented studies.

Differential misclassification occurs when the misclassification is not independent of the outcome or exposure. This bias can seriously distort the effect estimates. Multiple examples are discussed below as well as the likeliness of the occurrence in the presented studies in this thesis and the potential effects of these biases.

Immortal time bias

This bias occurs when there is a period of follow-up or observation time during which death cannot occur. This might happen for instance when patients need to have at least

a number of prescriptions before they enter the cohort and when this time is not properly taken into account (e.g. classified as exposed instead of unexposed)[23]. This form of immortal time bias might also occur when patients are classified based on a prescription they will get in the future. The time until the prescription is then immortal and is classified as exposed while patients are actually unexposed. Such a misclassification might then result in a lower relative risk of the studied drug[24].

Immortal time bias can only occur in cohort studies and may therefore only be an issue in Chapter 2.2, 2.4 and 2.7. Since patients were followed from the first prescription and all time between the start of follow-up and the first prescription for the drug of interest (either DPP4-I or GLP1-RA) was classified as unexposed, these studies do not suffer from this bias.

Recall bias

Recall bias is defined as a bias due to differences between what cases and controls remember about for instance exposure or events in the past[25]. One of the advantages of the use of EHDs is that data is collected prospectively and it often does not use self-reported data which excludes the possibility of recall bias. Therefore this has likely not been a problem in the studies presented in Chapter 2. However, potential confounders such as falls may have suffered from recall bias as these can only be registered after questioning the patients. We were only able to adjust for falls in the cohort studies (Chapter 2.2, 2.4 and 2.7). Consequently, information on falls was collected before the fracture occurred and this may have reduced the potential for recall bias.

Another variable which may be effected by recall bias is body mass index (BMI). It is possible that weight is based on self-reported weight by the patients instead of being measured by the general practitioner (GP) every time. This may then result in an underestimated BMI. However, when this occurred equally between the different exposure groups this results in a bias towards the null. If BMI was more underreported in the incretin exposure groups this may have masked a potential protective association between the incretin agents (either DPP4-Is or GLP1-RAs) and fracture risk.

Part of the Maastricht Study data is based on questionnaires and therefore recall bias might be an issue in the studies presented in Chapter 3. Chapter 3.1 is only based on collected pharmacy data, so recall bias is not an issue there. Chapter 3.2 only used self-reported data on the duration of disease. This may have been more accurately recorded in patients using metformin as the time since diagnoses of T2DM is likely to be shorter as compared to participants with T2DM using insulin. When insulin users underestimate the duration of disease this might result in an estimate which is not fully adjusted for disease duration. Disease duration is associated with poorer bone quality. Not fully adjusting for disease duration might therefore result in an effect away from the null. In contrast, when insulin users overestimate the disease duration over-

adjusting may occur, resulting in an effect towards the null. In Chapter 3.3 only metformin users were investigated, and therefore recall bias is not expected.

Prothopatic bias

This can arise when an underlying outcome (not yet diagnosed) causes the studied exposure[26]. A potential association where this could occur is when use of incretin agents and risk of pancreatic cancer is studied. Undiagnosed pancreatic cancer may result in T2DM or T2DM-like symptoms, which will lead to treatment with anti-hyperglycaemic drugs. Due to the pancreatic cancer the glycaemic control might worsen quite quickly resulting in the prescription of second-line treatment, such as incretin agents[27]. Then pancreatic cancer will be diagnosed. As a consequence, a false-positive result might be found, suggesting that these drugs increase the risk of pancreatic cancer. This is an example of reverse causality because the outcome of interest is actually causing the exposure (the prescription of incretin agents). A study investigating use of incretin agents and risk of pancreatic cancer showed a rapid increase in the risk of pancreatic cancer with only a small number of incretin prescriptions. The risk of pancreatic cancer declined with longer-term incretin use. One of the explanations for this increased risk with short-term use was prothopatic bias[28].

Prothopatic bias often arises if there is a long time between the onset of the disease and the time of diagnosis. When studying fracture as an outcome this will likely not be an issue except for vertebral fracture. It is estimated that only one third to one fourth of the vertebral fractures come to clinical attention[29,30]. Though, it is unlikely that a vertebral fracture will lead to prescription of anti-hyperglycaemic drugs. Additionally, we did not find an increased risk of vertebral fracture with current use of DPP4-Is or GLP1-RAs. It is unlikely that prothopatic bias has occurred in Chapter 3.2 and 3.3. To be an issue, worse bone quality, reduced bone strength or increased cfPWV should have caused an increase of the prescription of the investigated drugs.

Immeasurable time bias

Immeasurable time bias is a form of bias which occurs when an exposure is not measureable for a certain period of time due to for instance hospitalizations[31]. When this is different between exposed and unexposed groups or between cases and controls this will lead to bias. In a case-control study this might happen when outcomes like mortality are studied. It has been estimated that 53% of the patients in England dies in the hospital[32]. EHDs, such as the clinical practice research datalink (CPRD), only contain data on prescriptions prescribed by the GP. People who die are more often hospitalized in the period before, and during this hospitalization period the exposure is immeasurable. In cohort studies immeasurable time bias can occur when patients with a chronic disease, and consequently many hospitalizations, are compared to patients without the disease and a lower number of hospitalizations. Again the exposure is

immeasurable and this than might result in a virtual protective effect of the studied exposure.

There is no reason to suspect that patients are often hospitalized just before a fracture, so this will not influence the results of the case-control studies in Chapter 2.3 and 2.5. However, this type of bias might also have occurred when the date of fracture is not correctly recorded which will then result in immeasurable time. For the case-control studies presented in Chapter 2.3 and 2.5 we used Danish hospital data including the admission date[33]. Therefore it is expected that the date of fracture is accurate.

Additionally, in our cohort studies we compared person time of patients with the same disease and thereby limited the immeasurable time window bias in Chapter 2.2, 2.4 and 2.7[31]. For the studies presented in Chapter 3, pharmacy dispensing records were collected. We might have missed dispensings due to hospitalizations. However, this is not expected to be differential between the studied groups, and therefore immeasurable time bias is probably not an issue in these studies.

Time window bias

When the opportunity for exposure is different between cases and controls (in a case-control study), time window bias can occur[34]. This was shown in a study investigating the association between use of statins and lung cancer[35]. The end of study was determined by the date of the cancer diagnosis for the cases. For the controls it was set to the end of the study period. Consequently, the average observation period was longer for controls as for cases. The exposure was determined as having at least one statin prescription. This resulted in a higher chance of exposure for the controls and finally an artificial 40% reduced risk of lung cancer with use of statins.

In our case-control studies the index date for the controls was set to the date of fracture of the cases, avoiding this form of time window bias. Additionally, the mean duration of treatment with incretin agents, was similar for cases and controls, suggesting that they have had the same opportunity of exposure (Chapter 2.3 and 2.5)[34].

Missing data

The way missing data are handled may also result in bias. When the missingness is related to the outcome performing a complete-case analyses will result in a biased estimates. However, when the missingness is unrelated to the outcome a complete-case analyses will not result in biased estimates for the investigated exposure although a complete-case analyses will result in lower power and less precision due to the excluded observations. Another method to deal with missing data is by adding an indicator for the missingness to the analysis. This will always result in biased results as the correlation between the variables is ignored[36,37].

The studies using the Danish national health services register (NHSR) data did not suffer from missing data. For the CPRD data we had missing data for some lifestyle variables (BMI, alcohol use and smoking) and glycosylated hemoglobin A1c (HbA1c). For these analyses, we applied the indicator method, which might have resulted in a biased effect estimate. Since the results were comparable to the results from Chapter 2.3 and 2.5, this suggests that the amount of bias might have been limited. In addition, in Chapter 4.1 we showed that the different methods to handle missing data (complete-case analysis, adding an indicator or performing MI) resulted in comparable effect estimates in time-fixed analyses when combined with multivariate adjustments. However, it needs to be taken into account that these results were based on time-fixed analyses, while most studies in this thesis were analysed by use of a time-dependent approach.

In both Chapter 3.2 and 3.3 we have applied the complete-case analysis method which, under the assumption that the missingness is not related to the outcome, results in unbiased estimates. When the missingness was related to the outcome this may have resulted in biased results. In Chapter 3.3 most of the cases were excluded due to missing cfPWV data, which was mainly missing due to logistic reasons, and therefore not expected to cause bias.

Selection bias

Immortal time bias

When immortal time is excluded from the analyses, it is classified as selection bias[38]. An example includes a study in which unexposed person time was excluded from the analyses[39,40]. As a consequence, patients who experienced an event in that period were not taken into account in the analyses at all, and this resulted in biased estimates. It has been shown that such an exclusion may result in an artificial reduced risk[24].

This form of immortal time bias was not an issue in the cohort studies presented in this thesis (Chapter 2.2, 2.4 and 2.7), as the cohort entry was equal to the date of the first anti-hyperglycaemic prescription since start of the study. Additionally patients were classified as unexposed to the drug under study (DPP4-I/GLP1-RA) until their first prescription and this time was taken into account as unexposed and not excluded. As immortal time bias can only occur in cohort studies this was not an issue in the other presented studies in this thesis.

Depletion of susceptibles

Depletion of susceptibles can occur when prevalent users are compared to incident users and an acute unintended-effect is studied[27,41]. The prevalent users might have a

different risk for the adverse event than the first-time users, and this causes biased results. It is therefore important to compare new-users of such a drug with a potential acute adverse effect with new-users of the comparison drug.

Bone is continuously remodelled, the process of remodelling takes about 3-6 months. It has been shown that drugs which increase bone mineral density need to be taken for at least 6-12 months to prevent vertebral fractures[42] and more than 18 months to prevent other fractures including hip fractures[42,43]. It is therefore not expected that the effect of incretins on fracture risk is acute, and depletion of susceptibles based on this theory is therefore not an issue in Chapter 2.

Patients could also have an increased fracture risk just after they have started a certain type of anti-hyperglycaemic agents due to an increased fall risk. This is highly likely when patients start with insulin. However, this is unlikely with incretin agents, as they very rarely cause hypoglycaemic events[44]. In Chapter 2.7 a sensitivity analyses was performed only including new anti-hyperglycaemic drug users and this did not materially change the results. Chapter 3.2 and 3.3 used cross-sectional outcome data, therefore depletion of susceptibles was not an issue.

Time lag bias

Time lag bias occurs when patients with a certain disease are compared to patients with a different stage of the same disease[45]. When disease duration is associated with the outcome this results in bias. This bias may have occurred in the studies presented in Chapter 2.2-2.7 as we compared current use of incretin agents to use of other anti-hyperglycaemic drugs.

Duration of diabetes is positively associated with fracture risk[46]. The average disease duration is expected to be higher for current DPP4-I or GLP1-RA users than the users in the comparison group. Therefore, this might have masked a protective association between use of DPP4-Is or GLP1-RAs and risk of fracture. However, it has been hypothesized that duration of diabetes via increased complications of diabetes is associated with an increased risk of fracture[46]. We have adjusted the analyses of Chapter 2.2-2.7 for neuropathy and retinopathy and HbA1c (Chapter 2.2, 2.4 and 2.7) and this may have limited the bias by diabetes duration.

The results of the study in Chapter 3.2 may suffer from time-lag bias as well. We compared users of insulin to non-insulin users. The underlying disease severity may therefore be an alternative explanation for the results indicating that insulin users have worse bone micro-architecture and bone strength.

Another type of time lag bias occurs when the studied outcome has a long latency period[45]. Due to this long latency period an outcome will be more often detected in later stages of the disease, which makes it difficult to study the potential association between for instance first-line anti-hyperglycaemic treatment and risk of cancer as the latency period for cancer is long.

This form of time lag bias might have also played a role when studying fracture as an outcome. Incretin agents are second or sometimes even third-line (especially GLP1-RAs) treatment options. As a consequence the potential beneficial effect of incretin agents might have been shifted towards the null. However, there was no difference in the results between GLP1-RAs and DPP4-Is, while GLP1-RAs are more used as third than as second-line treatment.

Confounding by indication and channelling bias

Confounding by indication occurs when there is an extraneous determinant of the outcome parameters which is also associated with whether the drug of interest is prescribed or not[47]. This might for instance occur when the severity or the prognosis of the disease influences the choice of the prescribed drug. A large observational cohort study evaluating use of inhaled corticosteroids and risk of fracture showed that the initially found doubled fracture risk disappeared after adjusting for disease severity[48]. After adjustments the results in Chapter 2 changed only slightly, suggesting that confounding by indication might not have been a large issue. However, channelling bias may have been an issue.

Channelling bias is a form of bias which is specific to new drugs. When new drugs have claimed certain advantages, these new drugs might be selectively prescribed to patients with for instance a certain comorbidity[49,50]. The disease may than be attributed to the prescribed drug. When incretin agents entered the market it was hypothesized that they were associated with a decreased risk of fracture. Therefore incretins may have been preferably prescribed to patients with an increased fracture risk which then may have masked the reduced risk of fracture. However, in Chapter 2.7 a comparable study was performed with a longer follow-up, which reduced the potential for channelling bias. These results were similar to the results of the earlier studies, suggesting that channelling bias was not a large issue.

Confounding

Confounding plays a role in every observational study. When a study is not properly adjusted for confounding factors the estimated effect measures will be distorted. We were not able to adjust our results for life-style factors such as BMI, smoking and alcohol use in Chapter 2.3 and 2.5 as these were not available. Additionally we could not adjust the results for lab measurements, such as HbA1c. This might have resulted in confounded results. However, the results were similar to the results from Chapter 2.2, 2.4 and 2.7 were we used CPRD data including data on life-style variables and HbA1c.

Another confounding factor in the studies presented in Chapter 2.2-2.7 might be the fact that people who suffer from alcoholism are preferably prescribed incretin

agents as opposed to sulfonylurea derivatives (SUs). SUs in combination with alcohol increase the risk of hypoglycaemia, while this is less of an issue with incretin agents. Use of alcohol has also been associated with an increased risk of fracture[51]. Alcoholism is not very well captured in the used databases, and this might therefore have masked a potential beneficial effect.

Our studies may have suffered from time-dependent confounding, which occurs when potential confounders are affected by previous exposure. This happens for instance when the association between use of statins and cognition is investigated[52]. Cholesterol level is an indicator for statin use, which itself influences the cholesterol level. Cholesterol level is associated with both future cognitive status and future statin use. Cholesterol level is also a mediator as statin use affects the cholesterol level which then may influence future cognition. The cohort studies presented in Chapter 2.2, 2.4 and 2.7 may have suffered from this form of bias as we adjusted for HbA1c. HbA1c is often used in practice to decide to change or not change the current treatment for T2DM. In addition, HbA1c is influenced by the used anti-hyperglycaemic treatment.

The results of the studies presented in Chapter 3.2 and 3.3 are also prone to confounding. This is especially true for the results in Chapter 3.2 as the small sample size limited the possibility to adjust the results for confounding. The results might therefore be the consequence of confounding. Additional to these potential sources of confounding, observational studies always suffer from residual confounding, due to factors that are not present in the databases, or not known to cause confounding. Residual confounding may be less of an issue when using data from the Maastricht Study as extensive phenotype data are available for all participants, which might reduce residual confounding.

Another form of selection bias may have occurred due to the fact that especially in the UK, GLP1-RAs are only recommended in patients with a BMI ≥35[53]. High BMI has been associated with a decreased risk of hip, osteoporotic and radius/ulna fracture[54]. When we would have found a decreased risk of fracture this may have also been the consequence of confounding by BMI. However, we did not find a decreased risk of fracture. Moreover, this selective prescribing of GLP1-RAs to patients with a BMI ≥35 is not an issue when using the Danish NHSR data, as there is no such an restriction on BMI in the Danish guidelines[55,56]. The results from the Danish data and the CRPD data regarding the association between use of GLP1-RAs and risk of fracture were comparable, suggesting that this restrictive prescribing in the UK has not influenced our results.

Overall implications & future perspectives

The results of the studies presented in Chapter 2 showed no association between the use of incretin agents and risk of fracture. Additionally, long-term use of DPP4-Is was

also not associated with fracture risk. These findings may be of value for clinical decisions regarding treatment of T2DM patients and incretin agents might therefore even be preferred in patients with a high fracture risk over other anti-hyperglycaemic drugs. However, these data were limited to a median duration of 1.6 years of actual DPP4-I use. As patients with T2DM often have to use anti-hyperglycaemic drugs the rest of their life, this is still an area that needs further investigation, including both DPP4-Is and GLP1-RAs.

Additionally, more research studying the potential association between use of GLP1-RAs and fracture needs to be performed, as the number of patients and certain types of fractures were low in the studies presented in this thesis. Therefore we may have failed to show an association just because the power in our study was too low. Furthermore, research investigating whether there is indeed a difference between liraglutide and exenatide and fracture risk is required. Another way to study the potential effect of liraglutide and exenatide on fracture risk is by investigating the association between use of these drugs and bone mechanical parameters, which can be done within the Maastricht Study.

It can be concluded from the results of Chapter 3.1 that the data from the Maastricht Study could be used in studies assessing drug utilisation or studies assessing the relative risk of specific outcomes associated with drug use. Most of the collected data in the Maastricht Study is not present in EHDs as this extensive phenotyping does not occur in practice. The Maastricht Study data thereby enables the study of unique potential associations between drug use and potential unintended effects. However, care should be taken when examining outcomes associated with the use of respiratory medications since functional impairment may have biased participation of these subjects. To make optimal use of the Maastricht Study data and the large number of included participants (>7000 currently included, 10,000 intended to be included) the prescription data needs to be updated and needs to be collected for all participants.

The results from the small explorative study in Chapter 3.2 are of interest but need to be replicated in larger cohorts as well as in longitudinal studies before definite conclusions can be drawn. In addition, bone micro-architecture and strength parameters need to be compared to patients without diabetes and the clinical relevance of the architectural and strength parameters and the relationship with fracture risk in T2DM needs to be determined.

The trabecular bone score (TBS) is a novel texture parameter reflecting pixel gray-level variations in dual X-ray absorptiometry (DXA) images[57]. It has also been related to bone micro-architecture[57]. A first study comparing T2DM patients to controls showed that part of the increased fracture risk seen in patients with T2DM is captured in the lumbar spine TBS[58]. However, more research is needed to investigate whether these bone scores can be used to predict fracture risk, especially in T2DM. DXA data will be available in the near future within the Maastricht Study and this could be used to

investigate the association between the TBS and bone microarchitecture parameters measured by the HR-pQCT scan.

Based on the results from Chapter 3.3 we concluded that dosage and duration of metformin was not associated with cfPWV. Longitudinal data on cfPWV is needed to fully elucidate whether dosage and duration of metformin is associated with a potential beneficial effect on arterial stiffness.

Based on the results from our case study in Chapter 4.1 we concluded that applying propensity scores to time-fixed time-to-event analyses may give similar results as multivariable adjusted analyses. Replications are needed as well as more research evaluating time-dependent time-to-event analyses.

Missing data are an issue in almost every research, including the Maastricht Study. Currently almost all studies using data from the Maastricht Study perform a complete-case analysis resulting in the exclusion of a large amount of participants (almost 25% in Chapter 3.3). More research on how to make as optimal as possible use of the collected data is needed.

Final conclusion

In conclusion, we showed that both short-term and long-term use of DPP4-Is use was not associated with fracture risk in patients with T2DM. Also, short-term use of GLP1-RAs was not associated with fracture risk. These findings may be of value for clinical decisions regarding treatment of T2DM patients, especially those at high fracture risk.

We demonstrated that the dispensing data of the Maastricht Study was representative as compared to national drug reimbursement data. With use of these data we showed that use of insulin was associated with deteriorating effects on micro-architectural and bone strength parameters. In addition, we reported that dosage and duration of metformin was not associated with aortic stiffness as measured by cfPWV in middle-aged patients with T2DM.

From a methodological point of view it can be concluded that PSs combined with different methods to handle missing data in time-fixed analyses may give similar effect estimates as compared to multivariable adjusted analyses. In addition, when performing pharmacoepidemiological studies it is important to think thoroughly about the study design and choose the optimal design for the research question and to avoid potential (time-related) biases as much as possible.

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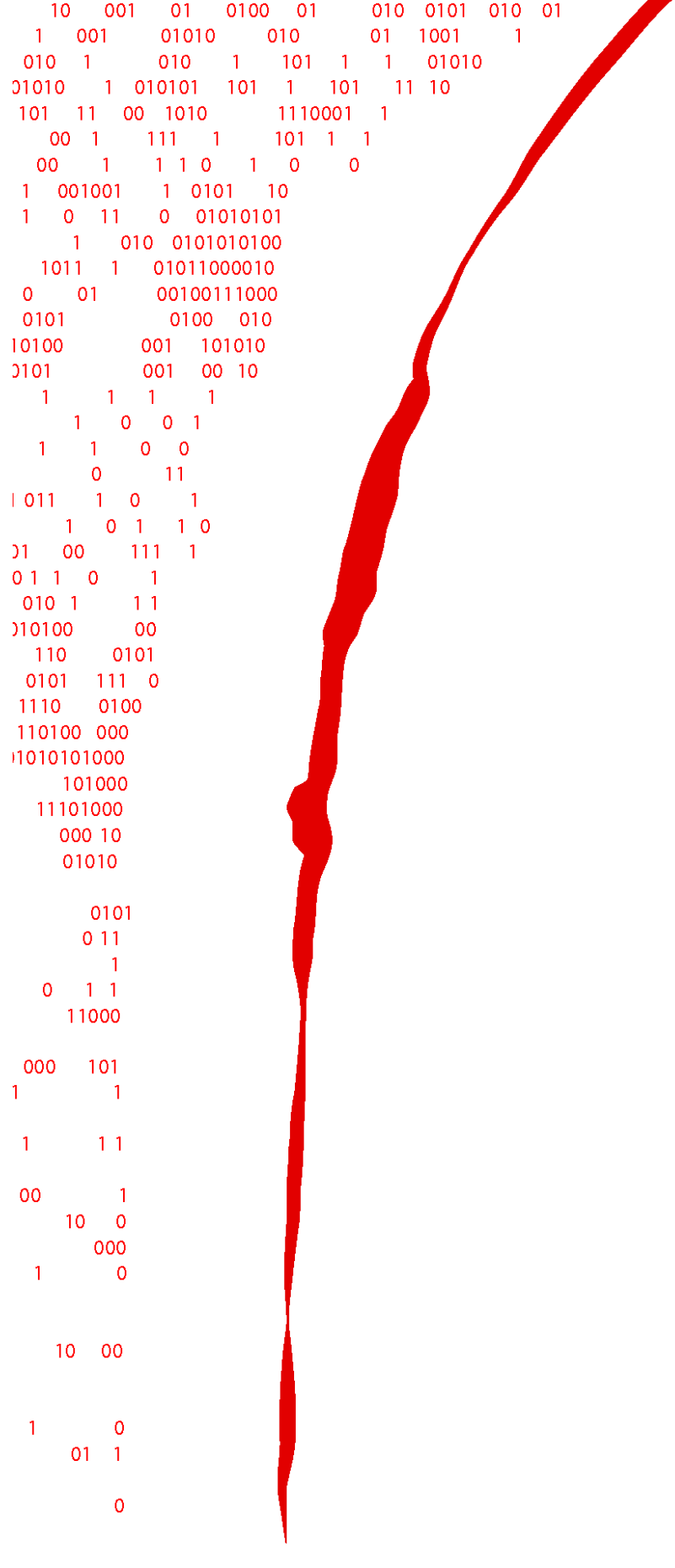

Appendices

Summary

Osteoporosis is defined as a systematic bone disease characterized by low bone mass and deterioration of the microarchitecture of the bone, leading to bone fragility and propensity to fracture. It is estimated that 22 million women and 3.5 million men suffered from osteoporosis in Europe in 2010. In particular, hip fracture has been associated with an increased risk of morbidity and mortality.

Diabetes is a chronic disease characterized by high blood glucose levels. The estimated total number of patients with diabetes (all types) world-wide was 171 million in 2000. Projections indicate that the number of patients will increase to 366 million in 2030. The estimated prevalence of diabetes in the Netherlands was 61.8 per 1000 women and 66.1 per 1000 men in 2014. About 90% of the patients with diabetes have type 2 diabetes mellitus (T2DM) which is characterized by insulin resistance and/or abnormal insulin secretion. The majority of patients with T2DM need anti-hyperglycaemic drugs to control their blood glucose levels.

Patients with T2DM have an increased risk of fracture as compared to patients without T2DM despite the fact that they often have a normal, or even increased, bone mass. It is therefore hypothesized that T2DM patients will have decreased bone quality and/or bone strength. In addition, an increased risk of falling and the use of certain anti-hyperglycaemic drugs (thizolidinediones) have also been associated with an increased risk for fracture.

Pharmacoepidemiology is the study of the use and effects of medications in large numbers of people. It frequently utilizes large “real-world” electronic healthcare databases (EHDs) that contains longitudinal data on patient characteristics, medical diagnoses, drug use and occasionally life-style variables such as body mass index (BMI), smoking status and alcohol use. These EHDs can be used to study rare adverse events which cannot be studied in randomised controlled trials (RCTs) due to the often restricted number of included patients.

Besides EHDs, prospective cohort studies can also be used to study potential adverse events. The Maastricht Study is a large observational prospective population-based cohort study which focuses on the aetiology, pathophysiology, complications and comorbidities of T2DM. Data on patients with and without T2DM is collected. In this thesis we used data on arterial stiffness, bone quality and bone strength.

When performing observational research one often has to deal with the following issues: missing data and confounding. Standard statistical techniques cannot handle missing data and delete all observations with missing values. Multiple imputation (MI) is a statistical technique which can be used to deal with missing data, by replacing the missing data with imputed values based on the observed data. Confounding occurs when there is a third factor which is associated with both the exposure and the outcome of interest, and distorts the potential association between the exposure and the

outcome. Adding this confounding factor to the analysis is a commonly used method to correct for confounding. Another way to deal with confounding is by use of propensity scores (PSs).

The overall objective of this thesis was to study the unintended effects of anti-hyperglycaemic drugs in population-based cohorts. This thesis consists of three main parts: 1) evaluation of the association between use of incretin agents, both dipeptidyl peptidase 4 inhibitors (DPP4-Is) and glucagon-like peptide 1 receptor agonists (GLP1-RAs), and risk of fracture; 2) an assessment of the usefulness of Maastricht Study data to evaluate unintended effects of diabetes medication, in particular of bone strength parameters and 3) to investigate the effect on treatment estimates when combining different techniques to handle missing data with PS analyses.

Chapter 2: Fracture risk and use of anti-hyperglycaemic drugs

In Chapter 2.1 the fracture incidence in Denmark in 2011 was studied and compared with estimations based on imputed data. The results showed that there were 80,760 incident fractures in 2011, of which 56.2% in women. The majority of the fractures occurred in the population aged 50 years and older (50,470) and the incidence rates of most fracture sites increased with age. The numbers of any and hip fracture were lower than the previously imputed estimates, whereas the number of forearm fractures was higher. Future research should therefore focus on how to improve those imputations as not all countries have nationwide registry data.

New anti-hyperglycaemic drugs, the incretin agents, have been marketed since 2007. They include DPP4-Is and GLP1-RAs. Both GLP1-RAs and DPP4-Is lower the blood glucose level by stimulation of the insulin secretion. GLP1-RAs do this directly by binding to a glucagon-like peptide-1 (GLP1) receptor which results into more insulin secretion. DPP4-Is bind to the enzyme which degrades GLP1, which leads to more available GLP1 and results indirectly in more insulin secretion.

A first meta-analysis of adverse events data of RCTs showed a 40% reduced risk of fractures with use of DPP4-Is. However, this meta-analysis only included a few RCTs and the total number of fractures was low. RCTs often apply strong in- and exclusion criteria which consequently result in a selected population which might give biased results. The potential association between use of incretins and fracture risk can also be investigated by performing a pharmacoepidemiological study. In such studies the use of drugs, in this case incretin agents, can then be studied in a larger group of patients. This is done in Chapter 2.2-2.7.

In both Chapter 2.2 and 2.3 the association between use of DPP4-Is and fracture risk was studied. In Chapter 2.2 data from Clinical Practice Research Datalink (CPRD) was used, which represents 6.9% of the United Kingdom population. In Chapter 2.3 we used

data from a Danish EHD. This EHD contains data on medical diagnoses as well as drug prescriptions of the Danish population. In contrast to the earlier published results we reported no association between use of DPP4-Is and fracture risk. In Chapter 2.4 and 2.5 use of GLP1-RAs and fracture risk was studied. We did not find an association between use of GLP1-RAs and fracture risk when using the CPRD data (Chapter 2.4) or using the Danish data (Chapter 2.5).

In Chapter 2.6 the results of Chapter 2.2-2.5 were combined using meta-analytic methods. After combining the results we did not identify an association between use of DPP4-Is or GLP1-RAs and fracture risk. In Chapter 2.7 the study period from Chapter 2.2 (2007-2012) was extended to 2007-2015 to investigate if the short duration of actual DPP4-I use, a limitation of the study in Chapter 2.2, was the reason we did not find an association. However, even with the longer duration of DPP4-I use the association between use of DPP4-Is and risk of fracture was not significant.

Chapter 3: Anti-hyperglycaemic drug use within the Maastricht Study population and potential unintended effects

In Chapter 3 we studied potential associations between use of anti-hyperglycaemic drugs and different outcomes measured within the Maastricht Study. We first investigated whether drug use in the Maastricht Study population was representative for the Dutch population (Chapter 3.1). This was studied by comparing drug use within the Maastricht Study to national data. Different drug classes were compared and the number of prescriptions per 1000 persons was comparable to national data, including for anti-hyperglycaemic drugs. However, the number of respiratory prescriptions was lower in the Maastricht Study population. In contrast, hypnotics and anxiolytics were higher in the Maastricht Study population as compared to national data. We concluded that drug use within the Maastricht Study was largely comparable to national data, and that the Maastricht Study data could be used to study drug - outcome associations.

In Chapter 3.2 bone quality and bone strength, as measured with high-resolution peripheral quantitative computed tomography, was compared between patient with T2DM who used and did not use insulin. Within the Maastricht Study HR-pQCT is used to study the micro-architecture of the distal radius, which can be used to estimate bone strength. The micro-architecture parameters, bone mineral density and bone strength were lower in insulin users, as compared to non-insulin users. This result remained even after correction for age, gender, body mass index, glycosylated hemoglobin A1c and duration of diabetes. Insulin use is most often used in a later stage of T2DM. Therefore it might be expected that patients using insulin have T2DM for a longer period or have a more severe form of T2DM than non-insulin users. Consequently, the results may be the consequence of the difference in disease duration, and severity, between the insulin users and the non-insulin users.

In Chapter 3.3 the association between dosage and duration of metformin use (an anti-hyperglycaemic drug) and arterial stiffness was investigated. Within the Maastricht Study, different measurements of arterial stiffness are performed as this is an important risk factor for cardiovascular diseases. One of the arterial stiffness measurements is the carotid to femoral pulse wave velocity (cfPWV). The cfPWV is determined by measuring the pressure waveforms between the carotid and femoral arteries. The higher the cfPWV, the stiffer the arteries. Previous studies suggested that metformin may have a beneficial effect on arterial stiffness. However, in this study we did not show an association between dosage and duration of metformin use and arterial stiffness as measured by cfPWV, in middle-aged patients with T2DM.

Chapter 4: Methodological Aspects

In Chapter 4.1 the effect on the treatment estimates was investigated when different techniques to handle missing data were combined with PSs. The results from our case-study showed that the estimates were quite similar when the different techniques were combined. However, we only investigated this in time-fixed analyses, in which the study period is seen as a whole. In pharmacoepidemiological studies, the study period is often split in short-time intervals to study the exposure in more detail (time-dependent analysis). Therefore, more research is needed to investigate the effect on treatment estimates, when different techniques to handle missing data are combined with PSs in time-dependent analyses.

General Discussion

In Chapter 5 the results of the different studies presented in this thesis are placed into a broader context. In addition, different forms of bias and confounding which may have occurred were discussed as well as the potential effects on the identified associations. This chapter concludes with a discussion of the clinical implications and prospects for future research.

Conclusion

In conclusion, we showed that both short-term and long-term use of DPP4-Is use was not associated with fracture risk in patients with T2DM. Also, short-term use of GLP1-RAs was not associated with fracture risk. These findings may be of value for clinical decisions regarding treatment of T2DM patients, especially those at high fracture risk.

We demonstrated that the dispensing data of the Maastricht Study was representative as compared to national drug reimbursement data. With use of these data we showed that use of insulin was associated with deteriorating effects on micro-architectural and bone strength parameters. In addition, we reported that dosage and

duration of metformin was not associated with aortic stiffness as measured by cfPWV in middle-aged patients with T2DM.

From a methodological point of view it can be concluded that PSs combined with different methods to handle missing data in time-fixed analyses may give similar effect estimates as compared to multivariable adjusted analyses.

Samenvatting

Osteoporose is een systemische botaandoening die gekarakteriseerd wordt door een lage bot massa en een verslechtering van de micro-architectuur van het bot wat resulteert in fragielere botten en een grotere kans op fracturen. Geschat wordt dat in 2010 ongeveer 22 miljoen vrouwen en 5.5 miljoen mannen osteoporose hadden in Europa. Met name heupfracturen zijn geassocieerd met een verhoogde kans op morbiditeit en sterfte.

Diabetes Mellitus is een chronische ziekte die gekenmerkt wordt door een verhoogde bloedglucose spiegel. Het geschatte aantal mensen met diabetes (alle types) wereldwijd was 171 miljoen in 2000 en de verwachting is dat dit stijgt naar 366 miljoen in 2030. De geschatte prevalentie van type 2 diabetes mellitus (T2DM) in 2014 was 61.8 per 1000 vrouwen en 66.1 per 1000 mannen in Nederland. Ongeveer 90% van de mensen met diabetes heeft T2DM, wat gekarakteriseerd wordt door insuline resistentie en/of abnormale insuline secretie. Het merendeel van de patiënten met T2DM wordt behandeld met bloedglucoseverlagende middelen om zo de bloedglucose spiegel zoveel mogelijk onder controle te houden.

Mensen met T2DM hebben een groter risico op het krijgen van een fractuur dan mensen zonder T2DM ondanks het feit dat ze vaak een normale of zelfs hogere botmassa hebben. Er wordt dan ook gedacht dat de kwaliteit en sterkte van het bot mogelijk verminderd is in patiënten met T2DM. Andere verklaringen zijn een verhoogd valrisico en ook het gebruik een bepaalde groep bloedglucoseverlagende middelen (thiazolidinediones) is geassocieerd met een toegenomen fractuur risico.

Farmacoepidemiologie bestudeert het gebruik en effect van medicijnen in grote aantallen mensen. Vaak worden hiervoor grote elektronische gezondheidszorg databases gebruikt die informatie bevatten over patiënt karakteristieken, medische diagnoses, medicatiegebruik en soms ook overige gegevens zoals lichaamsgewicht, roken en alcohol gebruik. Met deze grote gezondheidszorg databases is het onder andere mogelijk om bijwerkingen en effecten van medicijnen te onderzoeken die niet onderzocht kunnen worden in gerandomiseerde studies. Het aantal mensen dat geïncludeerd wordt in gerandomiseerde studies is vaak relatief laag waardoor bijwerkingen die minder vaak voorkomen niet/moeilijk onderzocht kunnen worden. Dit is echter wel mogelijk in observationele farmacoepidemiologische studies.

Naast het gebruik van grote gezondheidszorg databases kunnen ook prospectieve cohort studies gebruikt worden om mogelijke bijwerkingen van medicatie te onderzoeken. De Maastricht Studie is een groot prospectief cohort onderzoek dat zich met name op het ontstaan, complicaties en co-morbiditeiten van T2DM richt. Er worden zeer veel gegevens verzameld van gezonde personen en patiënten met T2DM. In dit proefschrift is gebruik gemaakt van gegevens over bijvoorbeeld vaatstijfheid en de botkwaliteit en -sterkte.

Bij het doen van onderzoek komt men vaak de volgende problemen tegen: hoe om te gaan met ontbrekende gegevens en wat te doen met factoren die het resultaat onbedoeld kunnen verstoren ('confounding'). Standaard statistische technieken kunnen niet omgaan met ontbrekende waarden en verwijderen dan ook alle observaties met ontbrekende waarden, waardoor veel gegevens verloren kunnen gaan. Een van de manieren om hiermee om te gaan is door het toepassen van 'Multiple Imputation', waarbij de ontbrekende gegevens geschat worden op basis van de aanwezige gegevens en zo alsnog alle observaties meegenomen kunnen worden in de analyses.

'Confounding' ontstaat wanneer er een derde factor is die zowel met de te onderzoeken determinant als de uitkomst is geassocieerd en de relatie tussen de determinant en de uitkomst verstoord. Het toevoegen van de mogelijk verstorende factor aan de analyse is de veel gebruikte manier om voor 'confounding' te corrigeren. Daarnaast kunnen ook 'propensity scores' gebruikt worden om te corrigeren voor 'confounding'. Er wordt dan per patiënt een 'propensity score' berekend die meegenomen wordt in de analyse.

Het doel van dit proefschrift is het bestuderen van onbedoelde effecten van bloedglucoseverlagende medicijnen in grote cohorten. Het proefschrift bestaat uit drie verschillende onderdelen:

1) Het bestuderen van de associatie tussen incretines, een bepaalde groep orale bloedglucoseverlagende medicatie, en het risico op een fractuur; 2) het onderzoeken van de bruikbaarheid van gegevens uit de Maastricht Studie om farmaco-epidemiologisch onderzoek uit te voeren en 3) het onderzoeken van het effect op de puntschattingen wanneer verschillende technieken voor het omgaan met ontbrekende gegevens gecombineerd worden met 'propensity scores'.

Hoofdstuk 2: Fractuur risico en het gebruik van bloedglucose-verlagende medicijnen

In Hoofdstuk 2.1 is de fractuurincidentie in Denemarken in 2011 onderzocht en vergeleken met schattingen op basis van geïmputeerde gegevens. De resultaten lieten zien dat er 80,760 nieuwe fracturen waren in 2011, waarvan 56.2% in vrouwen. De meerderheid van de fracturen werd gezien bij personen die 50 jaar of ouder waren (50,470) en de incidentie nam voor de meeste fractuur typen toe met de leeftijd. Het totaal aantal fracturen en het aantal heupfracturen was lager terwijl het aantal onderarmfracturen hoger was vergeleken met de geïmputeerde gegevens. Toekomstig onderzoek zou zich daarom moeten richten op het verbeteren van het imputeren van gegevens aangezien niet alle landen de beschikking hebben over grote gezondheidszorg gegevens om zelf incidenties te berekenen.

Sinds 2007 is er een nieuwe groep bloedglucoseverlagende middelen, genaamd incretines, beschikbaar gekomen. Binnen de incretines zijn er twee typen te onderscheiden, de dipeptidyl peptidase-4 remmers (DPP4-remmers) en de glucagon-

like peptide 1 receptor agonisten (GLP1-RAs). Zowel de GLP1-RAs als de DPP4-remmers zorgen voor verlaging van de bloedglucose door de afgifte van insuline te stimuleren. GLP1-RAs doen dit direct door aan de 'glucagon-like peptide -1' (GLP1) receptor te binden wat zorgt voor meer insuline afgifte. DPP4-remmers binden aan het enzym dat GLP1 afbreekt en zorgen er zo indirect voor dat er meer GLP-1 beschikbaar blijft wat vervolgens resulteert in een toename van insuline afgifte.

Een eerste meta-analyse, van bijwerkingen gerapporteerd in gerandomiseerde studies, liet een 40% lager risico op fracturen zien met het gebruik van DPP4-remmers. Echter, deze meta-analyse bevatte maar een beperkt aantal gerandomiseerde studies en totaal aantal fracturen. Gerandomiseerde studies vaak uitgevoerd in geselecteerde populaties wat kan leiden tot een vertekening van de resultaten. Een andere manier om de mogelijke associatie tussen het gebruik van incretines en fractuur risico te onderzoeken is door het uitvoeren van observationele farmacoepidemiologische studies. In zulke studies kan het gebruik van, in dit geval incretines, in een grotere groep gebruikers worden onderzocht. Dit is toegepast in Hoofdstuk 2.2-2.7.

In zowel Hoofdstuk 2.2 als Hoofdstuk 2.3 werd de associatie tussen het gebruik van DPP4-remmers en fractuurrisico bestudeerd. In Hoofdstuk 2.2 zijn gegevens van een grote huisartsen database, de 'Clinical Practice Research Datalink' (CPRD), representatief voor 6,9% van de Britse bevolking, onderzocht. In Hoofdstuk 2.3 zijn gegevens uit een Deense database bestudeerd. Deze Deense database bevat onder andere medische gegevens en medicatie gegevens van de gehele Deense bevolking. In tegenstelling tot hetgeen in eerdere onderzoeken werd gerapporteerd, toonden beide studies in dit proefschrift geen associatie tussen het gebruik van DPP4-remmers en fracturen aan. In Hoofdstuk 2.4 en 2.5 werd de relatie tussen het gebruik van GLP1-RAs en fracturen bestudeerd. Ook bij het gebruik van GLP1-RAs werd geen relatie gevonden op basis van de CPRD (Hoofdstuk 2.4) en de Deense database (Hoofdstuk 2.5).

In Hoofdstuk 2.6 zijn de resultaten van Hoofdstuk 2.2-2.5 samengevoegd door middel van een meta-analyse. Ook na het samenvoegen van de resultaten was er geen associatie tussen het gebruik van DPP4-remmers of GLP1-RAs en fractuurrisico. Vervolgens is in Hoofdstuk 2.7 de gebruikte studie periode uit Hoofdstuk 2.2 (2007-2012) verder uitgebreid naar 2007-2015 om te kijken of één van de beperkingen van de studie in Hoofdstuk 2.2, de korte duur van het gebruik van DPP4-remmers, de reden was dat we geen associatie vonden. Echter ook in de uitgebreide studie met langduriger gebruik van DPP4-remmers kon geen associatie tussen het gebruik van DPP4-remmers en fractuurrisico worden aangetoond.

Hoofdstuk 3: Bloedglucoseverlagend medicijn gebruik binnen de Maastricht Studie populatie en mogelijke onbedoelde effecten

In Hoofdstuk 3 zijn mogelijke associaties onderzocht tussen het gebruik van bloedglucoseverlagende medicijnen en verschillende uitkomsten gemeten in de

Maastricht Studie. In eerste instantie is onderzocht of het geneesmiddelgebruik in de Maastricht Studie populatie representatief was voor de Nederlandse populatie (Hoofdstuk 3.1). Dit is onderzocht door het medicijngebruik in de Maastricht Studie te vergelijken met nationale gegevens. Verschillende groepen medicijnen zijn vergeleken en voor de meeste groepen was het aantal prescripties per 1000 personen gelijk aan de nationale gegevens, inclusief de bloedglucoseverlagende middelen. Het aantal prescripties voor astma en chronische obstructieve longziekte was lager in de Maastricht Studie populatie. Daarentegen was het aantal prescripties voor slaap- en kalmeringsmiddelen juist hoger ten opzichte van landelijke gegevens. We concludeerden dat medicatie gebruik in de Maastricht Studie grotendeels representatief is voor de nationale populatie en dat Maastricht Studie gegevens gebruikt kunnen worden om mogelijke medicatie – uitkomst associaties te onderzoeken.

In Hoofdstuk 3.2 is de botkwaliteit en botsterkte, gemeten met behulp van 'high-resolution peripheral quantitative computed tomography' (HR-pQCT), vergeleken tussen patiënten met T2DM die wel en geen insuline gebruikten. Binnen de Maastricht Studie wordt met HR-pQCT gedetailleerd gekeken naar de micro-architectuur van het bot in de distale radius en kan onder andere de botsterkte berekend worden.

Zowel de micro-architectuur parameters als botdichtheid en botsterkte waren lager in de insuline gebruikers ten opzichte van de niet-insuline gebruikers, ook na correctie voor leeftijd, geslacht, 'body mass index', geglycosyleerd hemoglobine A1c en diabetes duur. Aangezien insuline gebruik bij patiënten met T2DM vaak de laatste stap in het behandelproces is, is te verwachten dat insuline gebruikers langer en mogelijk ook een ernstigere vorm van T2DM hebben dan niet-insuline gebruikers. De gevonden verschillen tussen de insuline en niet-insuline gebruikers in botkwaliteit kunnen het gevolg zijn van insuline gebruik maar kunnen mogelijk ook veroorzaakt zijn door het verschil in duur en ziekte-ernst (waarvoor niet goed kan worden gecorrigeerd in deze studie) tussen deze twee groepen gebruikers.

In Hoofdstuk 3.3 is de associatie tussen duur en dosis van metformine gebruik (een bloedglucoseverlagende medicijn), en arteriële stijfheid onderzocht. Binnen de Maastricht Studie wordt onderzoek gedaan naar verschillende maten van vaatstijfheid aangezien dit een belangrijke risicofactor is voor het ontstaan van hart- en vaatziekten. Als maat voor vaatstijfheid wordt onder andere gekeken naar de snelheid waarmee de drukgolf zich verplaatst over het traject van de halsslagader naar de liesslagader, de zogenoemde 'polsgolfsnelheid'. Hoe hoger deze snelheid hoe stijver de bloedvaten zijn. In eerdere studies werd gesuggereerd dat metformine een gunstig effect zou hebben op vaatstijfheid (metformine zou de arteriële stijfheid verlagen). Echter in de studie in dit proefschrift werd geen associatie gevonden tussen de dosis en duur van metformine gebruik en arteriële stijfheid, gemeten via de polsgolfsnelheid, in patiënten met T2DM van middelbare leeftijd.

Hoofdstuk 4: Methodologische aspecten

In Hoofdstuk 4.1 is het effect op de puntschatting onderzocht wanneer verschillende technieken om met ontbrekende waarden om te gaan gecombineerd worden met 'propensity scores'. Uit de resultaten van onze 'case-study' bleek dat er weinig verschil zat in de puntschattingen wanneer de verschillende technieken werden gecombineerd. We hebben dit echter alleen getest in een analyse waarbij we de studie periode als één geheel hebben gezien ('time-fixed analyses'). In farmacoepidemiologische studies wordt de studie periode vaak opgesplitst in kortere periodes om zo een nauwkeuriger beeld van de blootstelling te krijgen ('time-dependent analyses'). Er is daarom meer onderzoek nodig naar wat er gebeurd met de puntschatting wanneer technieken om met ontbrekende gegevens om te gaan gecombineerd worden met 'propensity scores' in deze 'time-dependent analyses'.

Algemene discussie

In Hoofdstuk 5 zijn de resultaten van de verschillende studies in een breder perspectief geplaatst. Daarnaast zijn er verschillende vormen van vertekening en verstoring ('bias' en 'confounding') besproken die mogelijk plaats hebben gevonden evenals het mogelijke effect op de gevonden associaties. Ook zijn de klinische implicaties van het onderzoek in dit proefschrift besproken, net als de mogelijkheden voor toekomstig onderzoek.

Conclusie

Op basis van dit proefschrift kan geconcludeerd worden dat zowel kort- als langdurig gebruik van DPP4-remmers niet geassocieerd is met fractuurrisico in patiënten met T2DM. Ook kortdurend gebruik van GLP1-RAs is niet geassocieerd met fractuurrisico. Deze bevindingen zijn mogelijk van belang bij het nemen van de beslissingen die betrekking hebben op de behandeling van patiënten met T2DM, en dan met name voor T2DM patiënten met een hoog fractuur risico.

Uit de onderzoeken binnen de Maastricht Studie kan geconcludeerd worden dat de medicatie gegevens van de Maastricht Studie representatief zijn ten opzichte van de nationale medicatie gegevens. Daarnaast werd een associatie gevonden tussen insuline gebruik en een verminderde botsterkte en -kwaliteit. De dosis en duur van metformine gebruik bleek niet geassocieerd te zijn met vaatstijfheid in patiënten van middelbare leeftijd met T2DM in de Maastricht Studie.

Ten slotte kan er vanuit een methodologisch perspectief geconcludeerd worden dat wanneer 'propensity scores' gecombineerd worden met verschillende technieken om met ontbrekende gegevens om te gaan in 'time-fixed analyses', dit vergelijkbare resultaten geeft ten op zichte van multivariabele gecorrigeerde analyses.

Valorisation Addendum

Knowledge valorisation refers to the "process of creating value from knowledge, by making knowledge suitable and/or available for social (and/or economic) use and by making knowledge suitable for translation into competitive products, services, processes, and new commercial activities" (adapted definition based on the National Valorisation Committee 2011:8). In this addendum the societal relevance of the results presented in this thesis will be discussed as well as the possibilities for valorisation of our results.

Healthcare problem

Osteoporosis is defined as a systematic bone disease characterized by low bone mass and deterioration of the microarchitecture of the bone, leading to bone fragility and propensity to fracture. It has been estimated that about 22 million women and 3.5 million men suffered from osteoporosis in Europe in 2010[1]. The worldwide annual estimated costs of hip fractures, both direct and indirect, were $34.8 billion in 1990 and are expected to increase to $131 billion in 2020[2].

Type 2 diabetes mellitus (T2DM) is a chronic disease characterised by high blood glucose levels. Estimations showed that about 422 million people suffered from diabetes (all types) in 2014[3]. Patients with T2DM have to take anti-hyperglycaemic drugs to control their blood glucose levels, often for the rest of their lives. This has a huge impact on patient's lives as well as on healthcare costs.

T2DM has been associated with an increased risk of fracture. Different mechanisms have been proposed which partly explain this increased risk, including an increased risk of falling, reduced bone strength or quality and the use of anti-hyperglycaemic drugs. However, the effect of recently introduced anti-hyperglycaemic drugs, the incretin agents, on fracture risk in the population at large was unclear.

Investigational approach

In this thesis the potential association between newly marketed drugs and fracture risk was investigated using "real-life" data. The class of incretin agents includes two different drug types, dipeptidyl peptidase 4 inhibitors (DPP4-Is) and glucagon-like peptide 1 receptor agonists (GLP1-RAs). The association between incretin agents and fracture risk was studied separately for the two types of incretins. In addition, the association was studied using two separate databases, one representative for the UK population and one representative for the Danish population. Thereafter the results were combined to further investigate the potential association between use of incretin agents and risk of fracture. This was followed by a study investigating long-term DPP4-I use (up to 8.5 years) and risk of fracture using the UK database.

Main findings

In contrast to the first results of a meta-analysis of adverse events data[4] we were not able to show an association between use of incretin agents, either DPP4-I or GLP1-RA, and risk of fracture in the population at large. In the different individual studies we did not found a reduced risk of fracture, with use of DPP4-Is or GLP1-RAs. In addition, when the results were combined in a meta-analysis, we were also not able to show a reduced risk of fracture. Moreover, when the duration of follow-up was extended we were still not able to show a reduced risk of fracture with long-term use (up to 8.5 years) of DPP4-Is.

Target population

The results of this thesis are important for general practitioners (GPs) making clinical decisions regarding treatment of T2DM patients, especially those at high fracture risk. Use of thiazolidinediones, another type of anti-hyperglycaemic drugs, has been associated with an increased risk of fracture. In patients at a high fracture risk those drugs are therefore not preferred. In this thesis it was shown that use of incretin agents was not associated with fracture risk. They may therefore be prescribed in patients with T2DM at high fracture risk, without increasing their fracture risk even further.

Based on the results of a first meta-analysis of adverse events data of clinical trials a 40% reduction of fracture risk with use of DPP4-Is was shown[4]. This may have led to the preferred prescribing of DPP4-Is as compared to other second-line treatment for T2DM patients. However, only a small number of studies were included and the total number of fractures was only 63. Based on the results from the presented pharmaco-epidemiological studies in this thesis it can be concluded that there is no association between use of DPP4-Is or GLP1-RAs and risk of fracture. There is therefore no reason to preferably prescribe DPP4-Is or GLP1-RAs to patients with T2DM based on the potential protective effect on fracture risk with these incretin agents.

Recently, updated meta-analyses, including a larger number of trials, have been published and reported no association between use of DPP4-Is and fracture risk[5,6]. This shows that a meta-analysis of adverse events data with only a small number of trials included could lead to biased results and should therefore be interpreted with caution.

When interpreting the results of pharmacoepidemiological studies potential sources of bias and confounding need to be considered, as these may have a large influence on the final results. However, when performed well, pharmacoepidemiological studies are relatively cheap option to study drug-outcome associations which can be used to prevent unnecessary expensive clinical trials investigating the same drug-outcome associations. In addition, with pharmacoepidemiological studies it is possible to investigate associations using data from real-life patients, which is expected to better represent clinical practice than the data of patients included in randomised controlled trials.

Innovation and future research

Although we showed that use of DPP4-Is and GLP1-RAs was not associated with fracture risk, more research is still needed. Especially the potential association between use of GLP1-RAs and fracture needs to be further studied. Long-term GLP1-RA use needs to be investigated as well as research investigating whether there is indeed a different association between the different GLP1-RA types (liraglutide and exenatide), and fracture risk.

With the studies in Chapter 3 we showed that drug dispensing data in combination with Maastricht Study data can be used to perform pharmacoepidemiological studies, with new unique parameters, such as bone strength and bone micro-architecture, which are often not available in electronic health care databases. This makes the Maastricht Study a very unique cohort and gives many opportunities for future research and valorisation of the results. For instance, data from the Maastricht study could be used to study the potential effects of anti-hyperglycaemic drugs on bone mechanical parameters.

Another option for future studies with use of data from the Maastricht Study is investigating the association between different bone strength and micro-architecture parameters and the trabecular bone score (TBS). This bone score is a novel texture parameter reflecting pixel gray-level variations in dual X-ray absorptiometry (DXA) images. DXA data will be available in the near future in the Maastricht Study which then could be studied in combination with bone strength and micro-architecture parameters as well as in combination with the use of different anti-hyperglycaemic drugs.

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Curriculum Vitae

Annemariek Driessen was born on November 7th, 1988 in Naarden, The Netherlands. She graduated from secondary school at Sint Vitus College Bussum in 2007. In August 2010 she obtained her Bachelor's degree in Biomedical Sciences at Leiden University. Hereafter she studied statistical sciences for the life and behavioral Sciences and obtained her Master's degree in Mathematics at Leiden University, September 2012.

In November 2012 Annemariek started her PhD research at the Department of Clinical Pharmacy and Toxicology of the Maastricht University Medical Centre+, within the School for Public Health and Primary Care (CAPHRI) at Maastricht University. This research was supervised by prof. dr. J.P.W. van den Bergh, dr. F de Vries, dr. R.M.A. Henry, and dr. H.A.W. van Onzenoort. During her PhD Annemariek also worked at the research center of the Maastricht Study were she participated in data collection.

Annemariek has been awarded with two young investigator awards during her PhD, and she has given several talks at international conferences. Currently she is appointed as a postdoctoral research fellow at Maastricht University within CAPHRI and the School of Nutrition and Translational Research in Metabolism (NUTRIM).