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Associations with fracture in patients with diabetes: a nested case–control study

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ABSTRACT

Objectives: Diabetes mellitus is associated with an increased risk of fractures, which is not fully explained by bone mineral density and common risk factors. The aim of this study is to investigate the association of medication and biochemical markers on the risk of fracture in a diabetes population.

Design and setting: A nested case–control study was conducted based on Danish diabetes patients from The Danish National Hospital Discharge Registry.

Participants: The cases of the study were diabetes patients with a fracture (n=24 349), and controls were diabetes patients with no fracture (n=132 349). A total of 2627 diabetes patients were available for an analysis of patient characteristics, comorbidities, biochemical parameters and drug usage.

Results: Age (OR=1.02, 95% CI 1.01 to 1.04), diabetes duration (OR=1.06, 95% CI 1.02 to 1.09), a diagnosis of previous fracture (OR=2.20, 95% CI 1.55 to 3.11), an alcohol-related diagnosis (OR=2.94, 95% CI 1.76 to 4.91), total cholesterol level (OR=2.50, 95% CI 1.20 to 5.21) and the usage of antiepileptics (OR=2.12, 95% CI 1.39 to 3.59) all increased the odds of fracture. Low-density lipoprotein cholesterol levels decreased the odds of fracture (OR =0.34, 95% CI 0.16 to 0.74), where the level of 3.04–5.96 mmol/L was optimal with regard to fracture risk.

Conclusions: Low-density lipoprotein cholesterol may improve our understanding of fractures in diabetes patients, and it may be added to current fracture risk models in diabetes patients.

Strengths and limitations of this study

- The Danish National Hospital Discharge Registry covers the entire Danish population and has high validity.
- Information on medication bought on prescription and clinically measured biochemical markers were available for a large group of patients with diabetes.
- We were unable to assess whether medication was actually taken and at which intervals it was taken; however, we assume that non-compliance was only an issue in a small proportion, and that most antidiabetic agents and diabetes-associated therapies were taken on a regular basis.
- This study was a retrospective case–control study with certain limitations, thus causality cannot be assessed.

INTRODUCTION

Diabetes mellitus (DM) is associated with an increased risk of fractures.[1 2] The OR of hip fracture has been shown to be 6.9 (95% CI 3.3 to 14.8) in type 1 diabetes and 1.4 (95% CI 1.3 to 1.5) in type 2 diabetes.[1] Owing to the high prevalence of type 2 diabetes in the general population, type 2 diabetes constitutes a large part of the total number of diabetes related fractures. Furthermore, bone mineral density (BMD) was found to be increased in type 2 diabetes, and decreased in type 1 diabetes. However, the decrease in BMD was not of a magnitude which can explain the increased fracture risk in type 1 diabetes.[1] The Fracture Risk Assessment Tool (FRAX) score, a tool to determine fracture risk by BMD and common risk factors, for determining 10-year fracture risk was less valid in diabetes patients.[3] Thus, common markers of bone frailty seem unable to detect and predict fractures in diabetes patients. Patients with diabetes may be more susceptible to falls due to hypoglycaemic events, orthostatic hypotension as an adverse effect to antihypertensive drugs, impaired vision and decreased sensation caused by retinopathy and neuropathy, foot ulcers and rapid fluctuations in plasma glucose.[4] Observational studies report an increased risk of fracture when adjusted by hypoglycaemic events, previous falls and diabetes complications.[5–7] Vestergaard *et al*[8] did in a different cohort from the The Danish National Hospital Discharge Register report decreased fracture risk in metformin and sulfonylurea users. Other studies report neutral outcomes with the use of metformin and sulfonylurea.[9–11] Glitazone use is reported to increase fracture risk in patients with

diabetes.[12 13] Previous observational studies have reported an increased risk of fracture with increasing HbA1c levels.[14 15] The increased fracture risk in diabetes seems to be entangled in complications, medication use and biochemical markers.

A previous observational study showed that low non-fasting high-density lipoprotein cholesterol (HDL) levels protected against fractures.[16] However, low-density lipoprotein cholesterol (LDL) was not reported in this study, which may influence the interpretation of the results. Another observation found that total cholesterol, but not LDL or HDL,[17] was a predictor of fracture risk, whereas, an additional study found no association of high total cholesterol and fractures.[18]

The aim of this study is to investigate whether medication use and routine biochemical parameters are associated with fracture risk in diabetes patients.

METHODS

The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement guideline for reports of case–control studies has been followed.[19]

Design

The study was conducted as a nested case–control study in a cohort of diabetes mellitus patients. The cases were diabetes mellitus patients with a subsequent fracture in the period 1 January 2008 to 31 December 2011. Controls were diabetes patients without a subsequent fracture in the same time period. A fracture before 2008 was considered as a previous fracture. Approval was obtained by the Danish Data Protection Agency.

Diabetes assessment and fracture assessment

We extracted data regarding all patients with DM from The Danish National Hospital Discharge Register in the time period 1 January 1977 to 31 December 2011 using International Classification of Diseases (ICD)-10 and ICD-8 codes (ICD-10 codes: E10-E14, ICD-8 codes: 249-250). The Danish National Hospital Discharge Register was founded in 1977 and covers all inpatient contacts from 1977 to 1994, and from 1995 also all outpatient visits to hospitals, outpatient clinics and emergency rooms. From the same register, we obtained incident fractures in the same time period using ICD-10 codes. (ICD-10 codes: S02.0–S02.9, S07.0–S07.9, S12.0–S12.9, S22.0–S22.9, S32.0–S32.8, S42.0–S42.9, S52.0–S52.9, S62.0–S62.9, S72.0–S72.9, S82.0–S82.9, S92.0–S92.9). In box 1 the fractures are grouped by site. The fractures were a sum of low-energy and high-energy fractures. It was possible to link a diagnosis of diabetes and diagnosis of fracture due to a unique personal identifier. The same unique identifier made it possible to link to the exposure variables below.

Exposure variables

For an overview of acquired exposure variables and their related ICD-codes, Anatomical Therapeutic Chemical Classification system (ATC) codes, and Nomenclature for Properties and Units (NPU) codes (see box 1). NPU codes are an international classification system to be used in Clinical Laboratory Sciences. From The Danish National Hospital Discharge Register data regarding comorbidities were obtained. From the Central Region of Jutland, biochemical markers (2008–2011) and medication use from the prescription registry (2008–2011) by NPU-codes and ATC-codes were collected. Only data regarding redeemed drugs on prescription and not over-the-counter drugs were available. Age was defined as the age of the individual at 1 January 2008. Episodes of hypoglycaemia were collected. We did not limit the users by a time window, as hypoglycaemic events may be increased at the start of a new drug, and decreased bone strength would follow a longer period of use. The duration of DM (DM duration) was defined by the time from the date of DM diagnosis to the end of the prescription register, 31 December 2011. Furthermore, from the Central Region of Jutland BMI, and dual-energy X-ray absorptiometry (DXA), t-scores were collected, however, these were only available for a small subgroup and not applied in the adjusted analysis. Data on biochemical markers were collected from the hospitals in the Central Region of Jutland, thus, the number of patients with information differs by marker. LDL was calculated by the Friedwald formula. The Friedwald formula is less accurate for triglyceride levels of more than 2.3 mmol/L, and not applicable when levels are more than 4.5 mmol/L.[20] We did not have data on smoking habits. Alcohol use was determined by the registration an alcohol-related diagnosis. A proxy variable for hypertension (yes/no) was created by drug usage; yes was defined as the use of diuretics, β-antagonists, calcium channel antagonists, ACE inhibitors/AT2 antagonists, antiadrenergic drugs with either peripheral or central effect, renin-antagonists, or hydralazine. A proxy variable for usage of drugs affecting bone was created by drug usage; yes was defined as the use of bisphosphonates, teriparatide, strontium ranelate, denosumab or hormone replacement therapy. Non-statin lipid-lowering drugs (fibrates, cholesterol absorption inhibitors, nicotinic acid and bile acid resins) were gathered in a composite exposure category.

Data regarding pharmaceutical drug usage were collected in 29 929 individuals. Any drugs bought were registered with ATC code, dose sold and date of sale for the period 1 January 2008 to 31 December 2011. Because all sales were registered to the individual who redeemed the prescription, the capture and validity of data are high. Biochemical data and medication data were excluded if these were subsequent to entering case status, thus, biochemical data and medication use reflect the state before fracture. Means were calculated

Box 1 Overview of included variables and respective codes from International Classification of Diseases (ICD), Anatomical Therapeutic Chemical Classification system (ATC), or Nomenclature for Properties and Units (NPU)

Diabetes mellitus and Fractures (ICD)
- Type 1 diabetes mellitus: DE100, DE101, DE109, 24 900, 24 909, 24 907, 24 906
- Type 2 diabetes mellitus: DE110, DE111, DE119, 25 006, 25 007, 25 009

Fractures
- Skull and facial bones: S02.0–S02.9, 800–804.99
- Neck: S12.0–S12.9
- Rib, sternum and thoracic spine: S22.0–S22.9, 807 809, 810
- Lumbar spine and pelvis: S32.0–S32.8, 808–808.09,808.11–808.19, 808.91–808.99, 809,
- Shoulder and upper arm: S42.0–S42.9, 811–812, 818–819
- Forearm: S52.0–S52.9, 813, 818–819
- Wrist and hand: S62.0–S62.9, 814–816.09, 816.19, 816.99–817, 818–819
- Femur: S72.0–S72.9, 820–820.12, 820.18–820.92, 820.98–821.22, 821.28–821.32, 821.38–821.92, 821.98–821.99, 827–829
- Lower leg and ankle: S82.0–S82.9, 822–824.03, 824.08–824.13, 824.18–824.93, 824.98–824.99, 827–829
- Foot: S92.0–S92.9, 825 826.01–826.19, 826.99–829.99
- Spine fractures (ICD 8): 805–806

Comorbidities (ICD)
- AMI (DI21, DI22, DI23, 41 009, 41 099), diabetes-related nephropathy (DE102, DE112, 24 902, 25 002), diabetes-related neuropathy (DE104, DE114, 24 903, 25 003), diabetes-related retinopathy (DE103, DE113, 24 901, 25 001), diabetes-related peripheral artery disease (DE105, DE115, 24 904, 24 905, 25 004, 25 005), heart failure (DI50, DI110, DI130, DI132, 42 709 –42 711, 42 719, 42 899), alcohol (DF10, 303)

Antidiabetic drugs, antihypertensive drugs, statins (ATC)
- Insulin (A10A), biguanides (A10BA), β-cell stimulating (A10BB), glitazones (A10BG), GLP-1 receptor agonists (A10BX) antihypertensive drugs (C09, C07A, C08, C02AB, C02AC, C02CA, C02DB, C03C, C03AA, C03D)

  statins (C10AA)

Antiosteoporotic agents (ATC)
- Hormone replacement therapy (G03), strontium ranelate (M05BX03), teriparatide (H05AA), bisphosphonates (M05BA), denosumab (M05BX),

Others (ATC)
- Antipsychotics (N05A), antiepileptics (N03A), antidepressants (N06A) glucocorticoids (H02AB)

Biochemical markers (NPU)
- Alat (19 651), alkaline phosphatase (19 655), creatinine (18 016), HbA1c DCCT (03 835), total cholesterol (01 566), LDL cholesterol (01 568), HDL cholesterol (01 567), triglycerides (03 620), sodium (03 429), potassium (03 230), thyroid stimulating hormone (03 577), 25 hydroxy vitamin D (10 267)

when more samples were available. The biochemical analyses were performed in an ISO standardised laboratory.

The study group

A total of 156 698 diabetes patients were collected; 29 929 of these had data on pharmaceutical use. All cases of nephropathy were excluded; 2627 diabetes patients had information on biochemical markers and a subset of these additional on thyroid stimulating hormones (TSH) and vitamin D status (n= 2412 and n= 1592; respectively). In total, 2627 diabetes patients were available for an analysis of patient characteristics, comorbidities, biochemical parameters and drug usage. Figure 1 depicts the selection of the patients.

Statistical analysis

The statistical analysis was conducted using the STATA V.8 statistics package. Pharmaceutical use was handled as (yes/no) and biochemical markers were handled as numerical values. An unadjusted case–control analysis, and an adjusted case–control analysis were performed using logistic regression. The adjusted analysis was performed to address potential confounding. The adjusted analysis included characteristics, pharmaceutical use and biochemical markers. All results are presented as OR. In the following, the expression risk will be used

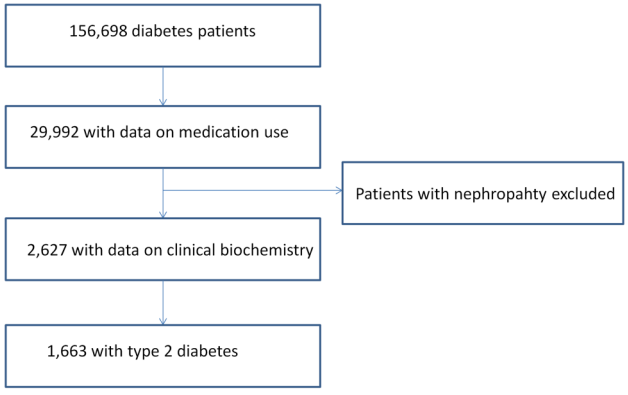

Figure 1 Flow chart of the selection of diabetes patients. Only patients with data on medication use and pharmaceutical use are included in the study.

synonymously with OR as less than 10% of the study group are cases. Subgroup analysis was performed on individuals who provided data on biochemical markers and pharmaceutical drug use. Specific analysis was performed on type 1 and type 2 diabetes patients. A sensitivity analysis was conducted on patients with type 2 diabetes excluding those with an alcohol-related diagnosis. The adjusted subgroup analysis included an analysis grouped by LDL levels in eight quantiles. To ensure robustness of the results, we performed an analysis by LDL level in tertiles and quartiles for the entire model. Statin duration was defined as the time between first redeemed prescription and the time of event (for cases), or before the end of follow-up, 31 December 2011 (for controls). Separate analyses were conducted using statin duration as a variable and excluding statin use as a variable.

RESULTS

A total number of 2627 diabetes mellitus patients were included in this study. A total of 175 (6.6%) of the diabetes patients had a subsequent fracture after the diabetes diagnosis. The 175 fractures were primarily located at the femur (19%), shoulder and upper arm (19%), forearm (17%), wrist and hand (12%), and knee and lower leg (12%), but also at the foot (5%), lumbar spine and pelvis (4%), rib, sternum and thoracic spine (4%), skull and facial bones (3%), and neck (2%). Table 1 gives an overview of evaluated characteristics, biochemical profile and pharmaceutical drug use of patients with and without fractures. The diabetes patients with fractures were significantly older, had a significantly longer diabetes duration, shorter duration of statin use, had a more frequent history of previous fracture and alcohol diagnosis, and cases were less frequent users of biguanides and glucagon-like peptide-1 (GLP-1) receptor agonists, and more frequent users of antidepressants and antiepileptics. Also, fewer of the patients with fracture used hydrophilic statins than patients without fracture, whereas, use of lipophilic statins did not differ between the groups. Patients with fractures were more susceptible to be users of loop diuretics compared to patients without fractures, whereas no difference was observed in thiazide use. Gender and diabetes complications were not significantly different between cases and controls.

Table 2 presents the results of the unadjusted analysis. In the unadjusted analysis, increasing age (1.02; 95% CI 1.01 to 1.03), increasing diabetes duration (1.04; 95% CI 1.01 to 1.07), previous fracture (2.05; 95% CI 1.48 to 2.82), an alcohol related diagnosis (3.04; 95% CI 1.95 to 4.73) and neuropathy (1.92; 95% CI 1.11 to 3.32) were all associated with increased risk of fracture. The only biochemical markers associated with fracture risk were HDL (1.54; 95% CI 1.09 to 2.17) and potassium (0.55; 95% CI 0.33 to 0.91). Usage of the antidiabetics biguanides (0.68; 95% CI 0.49 to 0.93), and glucagon-like peptide-1 receptor agonists (0.31 95% CI 0.12 to 0.86), were associated with decreased fracture risk. Increasing duration of statin use was also associated with decreased fracture risk (0.83; 95% CI 0.76 to 0.91).

Table 2 shows the results of the adjusted analysis. Increasing age (1.02; 95% CI 1.01 to 1.04), increasing diabetes duration (1.06; 95% CI 1.02 to 1.09), a previous fracture (2.20; 95% CI 1.55 to 3.11), and an alcohol-related diagnosis (2.94; 95% CI 1.76 to 4.91) preserves their significance. In this analysis, total cholesterol was associated with increased risk (2.50; 95% CI 1.20 to 5.21) and LDL (0.34; 95% CI 0.16 to 0.74) was associated with decreased risk of fracture significantly. Furthermore, potassium levels were associated with a decreased fracture risk (0.51; 95% CI 0.30 to 0.86). Antiepileptics were associated with increased fracture risk (2.12; 95% CI 1.33 to 3.59), whereas drugs affecting bone were associated with decreased fracture risk (0.50; 95% CI 0.27 to 0.95). Adjustment by statin duration instead of statin use did not alter significance of the results, with the exception of antihypertensive use, which was associated with increased fracture risk in this analysis (2.21; 95% CI 1.33 to 3.71). The adjusted analysis was performed specifically for type 1 and type 2 diabetes patients. LDL was still significantly associated with a decrease in fracture risk in the specific analysis for type 2 diabetes (selected results presented in table 2), and significance levels did not change. Too few type 1 diabetes patients were available for conducting a meaningful analysis (n=175). After exclusion of patients with an alcohol-related diagnosis, 1570 type 2 diabetes patients were available for analysis. In the fully adjusted model, LDL was not significantly associated with fracture (0.37; 95% CI 0.11 to 1.26). Likewise, total cholesterol was not significantly associated with fracture risk (2.41; 95% CI 0.74 to 7.80). Other variables did not change significance in this analysis.

LDL and fracture risk

In a stratified adjusted analysis specific to type 2 diabetes patients, the LDL level was divided by quantiles, and a significant association with increased risk of fracture was present for all quantiles when compared with the LDL level of 3.039–5.959 mmol/L. This reference group had a mean LDL level of 3.59 (0.52 SD). As statin use may influence the lipid levels, adjustment performed for statins was also included in the analysis (tables 2 and 3). Further adjustment by statin duration was performed, which did not change the results. Table 3 presents the relationship between LDL levels and fracture risk in type 2 diabetes patients.

Table 4 presents the association between LDL levels divided in tertiles and quartiles, and fracture risk in the 2627 diabetes patients. The highest quartile of LDL was associated with a decreased fracture risk, when comparing with the reference level of 2.21–2.66 mmol/L LDL, whereas, the other quartiles did not differ from the

Table 1 Baseline characteristics of the 2627 diabetes patients

Characteristic	Fracture after diabetes diagnosis (n=175) (95% CI)	No fracture after diabetes diagnosis (n=2452) (95% CI)
Age/years*	65.9 (63.8 to 68.0)	62.5 (61.9 to 63.0)
Diabetes duration/years*†	6.7 (6.1 to 7.3)	5.8 (5.6 to 6.0)
Sex male %	48.6 (41.1 to 56.0)	54.2 (52.2 to 56.2)
BMI kg/m^2 (n=153; 9 cases 144 controls)	27.2 (24.1 to 30.3)	29.5 (28.5 to 30.5)
DXA score		
DXA hip t-score* (n=200; 17 cases 183 controls)	−1.8 (−2.3 to −1.2)	−1.1 (−1.3 to −0.9)
Previous diagnosis		
Previous fracture %*†	36.6 (29.4 to 43.8)	22.0 (20.3 to 23.6)
Alcohol-related diagnosis %*†	15.4 (10.0 to 20.8)	5.7 (4.8 to 6.6)
T2D % (n=1860; 115 cases 1745 controls)†	85.2 (78.6 to 91.8)	89.7 (88.3 to 91.1)
Neuropathy %†	9.1 (4.8 to 13.5)	5.0 (4.1 to 5.8)
Retinopathy %	4.0 (1.1 to 6.9)	3.3 (2.6 to 4.1)
Vascular complication %	11.4 (6.7 to 16.2)	10.8 (9.6 to 12.8)
AMI %	16.0 (10.5 to 21.5)	13.4 (12.0 to 14.7)
Heart failure %*†	16.0 (10.5 to 21.5)	13.4 (12.0 to 14.7)
Biochemical parameters	**Mean (95% to CI)**	**Mean (95% CI)**
Alanine transaminase U/L†	34.0 (26.7 to 41.3)	33.7 (32.5 to 34.9)
Alkaline phosphatase U/L†	92.6 (79.6 to 105.5)	87.9 (85.3 to 90.4)
Creatinine µmol/L†	85.2 (80.4 to 89.9)	84.5 (83.0 to 85.9)
HbA1c (mmol/mol)	53 (52 to 55)	55 (54 to 55)
Total cholesterol mmol/L†	4.4 (4.3 to 4.6)	4.4 (4.3 to 4.4)
HDL mmol/L*†	1.4 (1.3 to 1.5)	1.3 (1.3 to 1.3)
LDL mmol/L†	2.2 (2.1 to 2.3)	2.3 (2.3 to 2.3)
Triglycerides mmol/L†	1.9 (1.7 to 2.0)	1.8 (1.8 to 1.9)
Sodium mmol/L†	138 (138 to 139)	139 (138 to 139)
Potassium mmol/L*†	4.0 (4.0 to 4.1)	4.1 (4.1 to 4.1)
Pharmaceutical use %	**(95% CI)**	**(95% CI)**
Insulin	29.7 (22.9 to 36.6)	28.5 (26.7 to 30.3)
Biguanides*	36.0 (28.8 to 43.2)	45.3 (43.3 to 47.3)
β-cell stimulants	24.0 (17.6 to 30.4)	25.0 (23.2 to 26.7)
Glitazones†	0.1 (0.1 to 0.3)	0.1 (0.1 to 0.1)
GLP-1 receptor agonists*†	2.3 (0.1 to 4.5)	6.9 (5.9 to 7.9)
Statins	58.9 (51.5 to 66.2)	56.7 (54.7 to 58.7)
Lipophilic statins	57.7 (50.3 to 65.1)	55.1 (53.1 to 57.0)
Hydrophilic statins*†	2.9 (0.4 to 5.4)	5.7 (4.8 to 6.6)
Statin duration (years)*†	1.4 (1.2 to 1.6)	2.0 (1.9 to 2.0)
Fibrates†	2.9 (0.4 to 5.3)	1.9 (1.4 to 2.5)
Nicotinic acid†	0.6 (0.1 to 1.7)	0.4 (0.2 to 0.7)
Cholesterol absorption inhibitors†	1.7 (0.1 to 3.7)	2.6 (1.9 to 3.2)
Bile acid resins†	0.6 (0.1 to 1.7)	0.4 (0.2 to 0.7)
Antihypertensives (any/none %)	63.4 (56.2 to 70.6)	60.6 (58.7 to 62.5)
Glucocorticoids	14.9 (9.5 to 20.2)	14.9 (13.5 to 16.3)
Diuretics*	46.3 (38.9 to 53.7)	37.6 (35.7 to 39.5)
Loop diuretics*†	32.6 (25.6 to 39.6)	22.1 (20.5 to 23.8)
Thiazides	18.9 (13.0 to 24.7)	21.4 (19.7 to 23.0)
Bone-affecting drugs†	7.4 (3.5 to 11.4)	10.1 (8.8 to 11.3)
Antidepressants*	28.6 (21.8 to 35.3)	22.1 (20.4 to 23.7)
Antipsychotics	5.1 (1.8 to 8.4)	5.8 (4.9 to 6.7)
Antiepileptics*†	13.7 (8.6 to 18.9)	7.0 (6.0 to 8.0)

*p<0.05 for difference between diabetes patients with incident fractures and diabetes patients without incident fractures.
†T test performed with unequal variances, due to estimation by Bartlett's test.
AMI, acute myocardial infarction; BMI, body mass index; DXA, dual-energy X-ray absorptiometry; GLP-1, glucagon-like peptide-1; HDL, high density lipoprotein; LDL, low density lipoprotein; T2D, type 2 diabetes.

Table 2 Unadjusted and adjusted OR for fracture after diagnosis of diabetes n=2627

Characteristic	Unadjusted OR (95% CI)	Adjusted OR (95% CI)†
Age (years)	1.02 (1.01 to 1.03)*	1.02 (1.01 to 1.04)*
Diabetes duration (years)	1.04 (1.01 to 1.07)*	1.06 (1.02 to 1.09)*
Sex: male	0.80 (0.59 to 1.08)	0.75 (0.52 to 1.06)
Previous fracture	2.05 (1.48 to 2.82)*	2.20 (1.55 to 3.11)*
Alcohol diagnosis	3.04 (1.95 to 4.73)*	2.94 (1.76 to 4.91)*
Neuropathy	1.92 (1.11 to 3.32)*	1.71 (0.94 to 3.10)
Retinopathy	1.20 (0.55 to 2.65)	0.95 (0.41 to 2.21)
Peripheral artery disease	1.06 (0.65 to 1.72)	0.73 (0.43 to 1.24)
AMI	1.23 (0.81 to 1.88)	1.16 (0.73 to 1.86)
Heart failure	1.11 (0.73 to 1.68)	0.87 (0.54 to 1.41)
Biochemical parameters		
Alanine transaminase U/L	1.00 (1.00 to 1.00)	1.00 (0.99 to 1.00)
Alkaline phosphatase U/L	1.00 (1.00 to 1.00)	1.00 (1.00 to 1.00)
Creatinine µmol/L	1.00 (1.00 to 1.00)	1.00 (0.99 to 1.00)
HbA1c %	0.89 (0.77 to 1.02)	0.92 (0.78 to 1.10)
Total cholesterol mmol/L	1.10 (0.92 to 1.30)	2.50 (1.20 to 5.21)*
Total cholesterol mmol/L n=1663‡	1.19 (0.96 to 1.49)	3.63 (1.25 to 10.5)*
HDL mmol/L	1.54 (1.09 to 2.17)*	0.56 (0.25 to 1.26)
LDL mmol/L	0.88 (0.70 to 1.10)	0.34 (0.16 to 0.74)*
LDL mmol/L n=1663‡	0.99 (0.75 to 1.34)	0.27 (0.09 to 0.81)*
Triglycerides mmol/L	1.03 (0.90 to 1.18)	0.83 (0.60 to 1.14)
Sodium mmol/L	0.96 (0.90 to 1.01)	0.98 (0.92 to 1.04)
Potassium mmol/L	0.55 (0.33 to 0.91)*	0.51 (0.30 to 0.86)*
Potassium mmol/L n=1663	0.68 (0.35 to 1.32)	0.67 (0.33 to 1.37)
Pharmaceutical use		
Insulin	1.06 (0.76 to 1.49)	1.03 (0.66 to 1.61)
Insulin n=1663‡	1.10 (0.65 to 1.85)	1.29 (0.67 to 2.49)
Glitazones	1.66 (0.38 to 7.22)	1.94 (0.38; 9.86)
Biguanides	0.68 (0.49 to 0.93)*	0.74 (0.49 to 1.10)
β-cell stimulants	0.95 (0.66 to 1.36)	1.06 (0.69 to 1.61)
GLP-1 receptor agonists	0.31 (0.12 to 0.86)*	0.36 (0.13 to 1.03)
GLP-1 receptor agonists n=1663‡	0.27 (0.07 to 1.10)	0.33 (0.07 to 1.49)
Statins	1.09 (0.80 to 1.49)	1.19 (0.73 to 1.94)
Statin duration (years)	0.83 (0.76 to 0.91)*	0.71 (0.63 to 0.80)§
Non-statin lipid lowering drugs	1.25 (0.64 to 2.44)	1.18 (0.55 to 2.51)
Antihypertensives (any/none %)	1.13 (0.82 to 1.55)	1.06 (0.62 to 1.80)
Glucocorticoids	0.99 (0.65 to 1.53)	0.86 (0.53 to 1.39)
Bone-affecting drugs	0.72 (0.40 to 1.28)	0.50 (0.27 to 0.95)*
Antidepressants	1.41 (1.00 to 1.99)*	1.25 (0.83 to 1.87)
Antipsychotics	0.88 (0.44 to 1.75)	0.50 (0.23 to 1.06)
Antiepileptics	2.11 (1.33 to 3.33)*	2.12 (1.25 to 3.59)*
Antiepileptics n=1663‡	1.75 (0.19 to 16.3)	1.64 (0.80 to 3.37)

Nephropathy cases were excluded from the analysis.
*p<0.05.
†Adjusted by all variables in the table excluding duration of statin use.
‡Analysis performed after excluding all type 1 diabetes patients (n=1663).
§Adjusted by all variables in the table excluding statins.
AMI, acute myocardial infarction; GLP-1, glucagon-like peptide-1; HbA1c, glycated hemoglobin A1c; HDL, high density lipoprotein; LDL, low density lipoprotein.

reference. The lowest and highest tertile of LDL did not differ in comparison with the middle tertile.

DISCUSSION

We found an association between risk of fracture and LDL-cholesterol for the whole cohort of diabetes patients as well as limited to type 2 diabetes. Nephropathy cases were excluded to eliminate bias from diabetic bone disease which range in a wide spectrum from adynamic bone disease to mixed renal osteodystrophy. Both low-energy and high-energy fractures were included in the analysis, as most fractures occur in non-osteoporotic individuals (T-score >−2.5).[21] The usage of LDL levels as a predictor of fracture risk was limited to type 2 diabetes patients, as results for type 1 diabetes patients are of uncertain quality due to wide CIs. An LDL level of 3.04–5.95 mmol/L was associated with

Table 3 Multivariate adjusted OR for a fracture in type 2 diabetes patients by LDL levels, n=1663

LDL mmol/L	N	Quantile	OR (95% CI)
0–1.54 *	207	1	3.13 (116 to 8.45)
1.54–1.82	208	2	0.42 (0.14 to 1.23)
1.82–2.06	208	3	0.78 (0.34 to 1.81)
2.06–2.23	208	4	0.40 (0.16 to 1.03)
2.23–2.44	209	5	Reference
2.44–2.67*	207	6	0.39 (0.17 to 0.89)
2.67–3.04*	208	7	0.31 (0.13 to 0.74)
3.04–5.96*	208	8	0.06 (0.015 to 0.25)

*p<0.05.
The model is adjusted by age, diabetes duration, sex, previous fracture, alcohol diagnosis, nephropathy, retinopathy, neuropathy, peripheral artery disease, AMI, heart failure, alat, alkaline phosphatase, creatinine, HbA1c, total cholesterol, HDL cholesterol, triglycerides, sodium, potassium, Insulin, biguanids, β-cell stimulants, GLP-1 receptor agonists, statins, non statin lipid lowering drugs, antihypertensives, glucocorticoids, bone affecting drugs, antidepressants, antipsychotics and antiepileptics. Type 1 diabetes patients and cases of nephropathy excluded.
AMI, acute myocardial infarction; GLP-1, glucagon-like peptide-1; HbA1c, glycated hemoglobin A1c; HDL, high density lipoprotein; LDL, low density lipoprotein cholesterol.

reduced fracture risk compared to lower LDL levels. The lowest LDL levels (0–1.5 mmol/L) were associated with an increased risk of fracture compared to higher levels. Thus, an LDL level of 3 mmol/L or more was associated with a decreased fracture risk. This was validated in a robustness test dividing LDL levels by quartiles, whereas the association was attenuated when dividing to tertiles. This may point at an optimal level of LDL in regard to fracture risk. This conflicts with current guidelines on LDL lowering in type 2 diabetes patients. The present American Diabetes Association (ADA) recommendations for LDL aim for levels at <1.8 mmol/L for participants with prior cardiovascular disease (CVD) and <2.5 for participants not having CVD.[22] A review concluded that LDL lowering was beneficial for all but the lowest-risk type 2 diabetes patients in terms of CVD, though the optimal target value was not firmly established, and further research is needed.[23] This study indicates that the CVD-preventive goal may increase the risk of fracture. This potential side effect should be assessed further in clinical trials determining the optimal target value of LDL in diabetes patients.

Table 4 Multivariate adjusted OR for a fracture in diabetes patients by LDL levels in tertiles and quartiles, n=2627

LDL mmol/L	N	Tertile	OR (95% CI)
0–1.95	876	1	1.31 (0.84 to 2.04)
1.95–2.48	876	2	Reference
2.48–6.39	875	3	0.76 (0.45 to 1.28)
LDL mmol/L	**N**	**Quartile**	**OR (95% CI)**
0–1.81	657	1	1.14 (0.65 to 2.00)
1.81–2.21	657	2	0.64 (0.39 to 1.05)
2.21–2.66	657	3	Reference
2.66–6.39*	656	4	0.45 (0.25 to 0.81)

*p<0.05.
The model is adjusted by age, diabetes duration, sexprevious fracture, alcohol diagnosis, nephropathy, retinopathy, neuropathy, peripheral artery disease, AMI, heart failure, alat, alkaline phosphatase, creatinine, HbA1c, total cholesterol, HDL cholesterol, triglycerides, sodium, potassium, Insulin, biguanids, β-cell stimulants, GLP-1 receptor agonists, statins, non statin lipid lowering drugs, antihypertensives, glucocorticoids, bone affecting drugs, antidepressants, antipsychotics and antiepileptics. Cases of nephropathy excluded.
AMI, acute myocardial infarction; GLP-1, glucagon-like peptide-1; HbA1c, glycated hemoglobin A1c; HDL, high density lipoprotein; LDL, low density lipoprotein cholesterol.

Increasing diabetes duration and neuropathy were associated with increased fracture risk suggesting that diabetes severity was a determinant of fractures. In the population studied, neither retinopathy nor neuropathy was significantly associated with fracture in patients with diabetes. This is in line with a previous observational study, where the fracture risk in patients with diabetes was not associated with either neuropathy or retinopathy.[7] Loop diuretics, but not thiazides, were more frequently used among patients with fracture compared to patients without fracture. This is consistent with previous findings.[24 25] Furthermore, diabetes patients with fracture were less prone to be biguanide and GLP-1 receptor agonist users. The beneficial effect of biguanide and GLP-1 receptor agonist users may be explained by the insignificantly higher number of type 1 diabetes patients in the fracture group, as these are more prone to fractures. Also, caution should be taken when interpreting the GLP-1 receptor agonist as it came on the market in 2007 and only short-term use can be evaluated. Further investigations of the effects of severity, duration and treatment of diabetes are needed.

Compared with other studies, this study may differ in the proportion of patients with type 1 DM, age and diabetes duration, incidence of retinopathy, metabolic control and use of antidiabetic drugs, which may have affected outcomes.[6 7 26] A relatively large percentage of the diabetes patients had a previous alcohol-related diagnosis. The percentage was larger for those with a fracture (15.4%) than those without (5.7%). Adjustment was performed for the alcohol-related diagnosis, as it both may relate to diabetes and to fracture, and thus, confound the results. When excluding patients with an alcohol-related diagnosis, LDL was not significantly associated with fracture risk in type 2 diabetes patients, however, a strong trend was present. This suggests that the observed association between LDL and fracture may be caused by alcohol use and related liver disease as these are associated with fractures and low LDL levels.[20 27 28] Furthermore, diabetes is associated with development of liver disease.[29] However, we observed no association between biomarkers for liver and fracture risk. Further research is needed to determine whether LDL in regard of fracture is a marker of liver dysfunction.

LDL may have a direct effect on bone. In vitro studies assessed the effect of LDL on bone cells; LDL-depletion

in the culture medium was found to inhibit the formation of tartrate-resistant acid phosphatase multinucleated cells (osteoclast-like cells). The effect was abrogated with the addition of LDL to the suspension.[30] The effect of LDL on osteoclasts was supported by an additional in vitro study, which found LDL to extend the survival of osteoclast-like cells, whereas HDL induces apoptosis.[31] In addition, a study found the osteoclast-associated receptor, which is a costimulator of osteoclast differentiation, to be induced by oxidised LDL in endothelial cells; however, the effect was abolished by specific inhibition of the nuclear factor of activated T-cells.[32]

An LDL receptor was found in osteoblasts.[33] Human osteoblasts were found to undergo apoptosis when exposed to native and oxidised LDL. However, this study did not determine at which level LDL kills osteoblasts.[34] A possible pathway of increased fracture risk with low LDL levels may also be found in the WNT-pathway. The low-density lipoprotein receptor-related proteins (LRP) 5, 6 and 8 were identified as positive regulators of the WNT-pathway and osteoblast differentiation.[35] LDL binds to the LRP through apolipoprotein B and apolipoprotein E moieties,[36] and may, therefore, stimulate the WNT-pathway. Cases of LRP 5 gene mutations with loss of function present with bone loss as well as cases of LRP 5 mutations present with abnormal glucose metabolism (diabetes and impaired glucose tolerance).[37] In addition LRP 5 mutations may also affect lipid metabolism.[37] This highlights the importance of a functioning LRP system. Consequently, too low concentrations of LDL may lead to impaired bone formation due to low WNT stimulation, which may explain the associations found in this study. We found no association between statin usage and fracture risk, however, increasing statin duration was associated with decreased fracture risk even when adjusting by LDL. The randomised JUPITER trial found no beneficial effect of statins and even a trend towards more fractures in the treated group.[38] It highlights the potential dual effects of statins on bone: a beneficial effect through the mevalonate pathway[39] and a negative effect through the LRP5 pathway.[35]

The strengths of this study were that we evaluated a large group of diabetes patients and performed multiple adjustments by patient characteristics, comorbidities, biochemical markers and pharmaceutical drug use. The fully adjusted analysis revealed strong associations with potentially large clinical implications.

However, this study was a retrospective case–control study with certain limitations, thus, causality cannot be assessed. This study may be influenced by selection bias, as biochemical markers only were available in a subpopulation. Sixty seven per cent of the diabetes patients were classified uniformly type 1 or type 2 diabetes, as some had an unspecified type and others were classified as both type 1 and type 2. The misclassification of diabetes diagnosis may limit our results. However, it may also reflect the difficulty in distinguishing type 1 and type 2 diabetes at the time of diagnosis. It is likely that 90% of the unspecified diabetes type is type 2 diabetes due to the population distribution. We were not able to distinguish between low-energy and high-energy trauma. This may affect our results, as high-energy trauma may be caused by unpredictable factors that do not relate to patient characteristics, medication use or biochemical markers. The inability to assess whether medication was actually taken, and at what intervals it was taken, is a limitation to the study. However, we assume that non-compliance was only an issue in a small proportion, and that most antidiabetic agents and diabetes-associated therapies were taken on a regular basis. The population was, in general, well regulated with HbA1c values within a narrow range. The tight glycaemic regulation supports good compliance with antidiabetic drugs. We also collected fall diagnoses, but few cases were recorded, so this did not change the results as only five of the 2627 patients had a prior diagnosis of tendency to falls, and none of these experienced a fracture. Previous studies show that the fracture risk is independent of previous falls.[5 6] Subgroup analysis of LDL was performed by eight quantiles (n=207–209). Biochemical markers, pharmaceutical use, BMI and DXA hip t-score data were only available for a limited number of participants in the study. BMI was previously shown not to be significantly associated with LDL, and the lack of adjustment should thus not affect the results.[40] Furthermore, important confounders like alcohol were evaluated through proxy variables. No data were available on smoking, and proxy variables performed poorly and were not used. Hormonal substitutions as growth hormone therapy and thyroid hormone therapy were not included in the analysis. However, TSH was evaluated in the analysis, and few participants were expected to receive growth hormone therapy. To our knowledge, we are the first to report an association between LDL and fracture risk in type 2 diabetes. The findings should be followed by clinical trials to decide whether LDL control should be performed in type 2 diabetes patients to prevent fractures and/or added to FRAX score in diabetes patients. In conclusion, the LDL level of 3.04–3.59 mmol/L was associated with a reduced risk of fracture compared to lower LDL levels.

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