The University Of Sheffield.

Thesis Title

Fracture risk in diabetes: insights from a study of bone microarchitecture

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A thesis submitted for the degree of

Doctor of Philosophy

The University of Sheffield

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Submission date 23rd October 2019

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Thesis abstract

Previous literature suggested an increase in the risk of fractures in diabetes. The risk seemed to be higher in T1D than in T2D but the effect of several features such as fracture site, gender, age, BMI and diabetes-related features such as DM duration, insulin use and the presence of complications has not been fully explored. This thesis investigated the risk of fractures in diabetes. The first meta-analysis (chapter 3) investigated the risk of hip and non-vertebral fractures in diabetes and how this risk was affected by several features associated with the patients and the disease. A significant increase in the risk of fracture in diabetes was found both for hip (RR 1.52, 95% CI 1.42-1.63) and for non-vertebral fracture (RR 1.20, 1.14-1.27). The increase in the risk was greater for insulin users and longer diabetes duration, at both sites. At the hip, the risk was higher in the younger population, women, and those with T1D. The second meta-analysis (chapter 4) investigated the risk of peripheral fractures in diabetes, since the wrist and ankle are the sites assessed by HR-pQCT. There was a discordant pattern and while at the wrist the risk of fractures was decreased (RR 0.85 95% CI 0.77 – 0.95) at the ankle the risk was increased (RR 1.30 95%CI 1.15 – 1.48). The sample included mainly T2D participants and the pattern was similar to the risk pattern observed in obesity.

Finally, a clinical study was conducted to assess axial DXA and peripheral microarchitecture in patients with type 1 diabetes with and without neuropathy. HR-pQCT was used to evaluate the standard site and also a less distal (14% limb length) site. There was no difference in DXA at lumbar spine or proximal femur between the groups. On HR-pQCT, the 14% site showed preserved trabecular structure particularly in the group without neuropathy and no abnormalities in the cortical compartment in the diabetic groups. At the standard site, cortical porosity was increased in the group with diabetes and neuropathy at the tibia. However, there were no differences in bone strength estimated by finite element analysis. Since bone turnover markers are decreased in diabetes, bone turnover is suppressed. This could suggest that the bone turnover suppression could prevent bone loss and preserve trabecular microarchitecture. Conversely, cortical porosity was increased only at the tibia in the group

with neuropathy. This finding suggested that vascular and/or neural integrity might also be important to bone remodelling and consequently, bone microarchitecture.

In summary, there was an increase in the risk of hip, non-vertebral and ankle fractures and a decrease in the risk of wrist fracture in diabetes. Our findings suggested that bone microarchitecture is not the main determinant of this increase in the risk of fractures.

Acknowledgements

I want to thank Prof Richard Eastell not only for the opportunity to come to Sheffield to work on research but also for all the teachings, guidance and encouragement during this project. I have learned a lot during this great experience. I extend these thanks to Dr Jennifer Walsh, my second supervisor. You have been both very supportive and inspiring.

I would like to thank the AUBM team, for always being helpful and supportive. I am especially thankful to Dr Margaret Paggiosi for her kind help in all sorts of applications, technical help with imaging issues and continuous encouragement. I would like to thank Dr Fatma Gossiel for her time and advice, and Gill Higginbottom for being always helpful and efficient. I want to thank the CRF team, especially Sister Julie Walker, Sister Angela Green, Sister Joanne Jackman and Jill Thompson for the joyful support during the clinical project. I also want to thank the Diabetes Unit team and the Diabetes consultants for their valuable support during the recruitment of participants. I would like to thank the AUBM colleagues for the lovely experiences.

I would like to thank the volunteers for their trust, their time and their invaluable contribution to the project.

I would like to thank Marian Schini, Sujatha Gopal, Stefania Pezzoli and Serena Vicario for being companions in this journey, friends for the good and hard moments. We shared doubts, anxieties, fears, complaints, uncountable laughs and maybe a few tears. Your friendship and support made this journey easier and special.

Finally, I am always grateful to my family for their unconditional love and support. Your reassuring presence in the background, no matter where or when, helps me to go further.

Contributions

Systematic review and meta-analysis on the risk of hip and non-vertebral fractures in diabetes (chapter 3)

The systematic review protocol was designed by School of Health and Related Research (ScHARR). I took part on the protocol discussion and approval with Prof Eastell.

The literature research and the first paper selection (based on title and abstract) was also done by ScHARR. For the selection of the systematic review to be updated, ScHARR conducted the research and selection of the review, that was discussed with me and Prof Eastell.

I designed and piloted the data extraction form under ScHARR's supervision.

Marian Schini helped with the selection of the original papers, data extraction and checking. Each of us downloaded half of the papers selected for full text assessment, assessed half of them for inclusion and checked the half assessed by the other one. Each of us extracted the data from half of the papers and checked the other half data extraction done by the other one.

For the systematic review, I analysed all the data under ScHARR's supervision.

For the systematic review data extraction, analysis and report I was supervised by Sue Harnnan from ScHARR.

For the meta-analysis, I analysed all the data.

The whole process was supervised by Prof Richard Eastell.

Systematic review and meta-analysis on the risk of wrist and ankle fractures in diabetes (Chapter 4)

I conducted all phases of the project, including the design of the protocol, study selection, data extraction and analysis and report under Prof Eastell's supervision.

An evaluation on bone microarchitecture in type 1 diabetes (Chapter 5)

I wrote the original protocol for the T1D and bone study and subsequent amendments. Dr Walsh and Prof Eastell supervised the clinical aspects of the study related to bone and Dr Dinesh Selvarajah supervised the clinical aspects related to diabetic neuropathy.

I acquired ethical and Research and Development approvals for the study and was responsible for site file maintenance, submission of amendments and general study management.

I wrote the participant information sheets, consent forms and other supporting documentation. I was responsible for study recruitment; I attended diabetes clinics, sent emails or letters across the hospital trust and University of Sheffield and to potential volunteers lists. I screened all volunteers prior to recruitment, wrote all study invitation and appointment letters and prepared the participant documentation packs. Letters were posted by Jill Thompson.

I booked and carried out all participant visits; I took consent from participants and performed all anthropometry and neurophysiologic function tests. I was responsible for coordinating participant travel, expenses, screening blood requests and study visits.

I completed all paper-based data collection forms and was responsible for managing the participant source notes. I completed all data entry.

All DXA and HR-pQCT scans were performed by Dr Margaret Paggiosi. Dr Paggiosi established the scanning protocols and obtained Medical Imaging and Medical Physics approval for the study. Scan analysis and finite element analysis was performed by Dr Paggiosi. Dr Paggiosi also provided images to be included in this thesis.

Screening blood tests were carried out by the Clinical Chemistry and Haematology Departments, Sheffield Teaching Hospitals, Sheffield.

I performed the statistical analysis for the study.

Dr Walsh and Professor Eastell provided guidance and direction throughout this work.

Communications

Publications

Vilaca T, Walsh J, Eastell R Discordant pattern of peripheral fractures in diabetes: a meta-analysis on the risk of wrist and ankle fractures.

Osteoporos Int. 2019 Jan;30(1):135-143. doi: 10.1007/s00198-018-4717-0.

Walsh JS, Vilaca T.

Obesity, Type 2 Diabetes and Bone in Adults.

Calcif Tissue Int. 2017 May;100(5):528-535. doi: 10.1007/s00223-016-0229-0. Review.

Poster Presentations

Vilaca T, Walsh J, Eastell R

A meta-analysis of the risk of ankle and wrist fractures in diabetes- American Society for Bone and Mineral Research (ASBMR) annual meeting 2017 7-11 September 2017 Denver, Colorado USA

Vilaca T, Walsh J, Eastell R

A meta-analysis of the risk of ankle and wrist fractures in diabetes - European Calcified Tissue Society (ECTS) Meeting 2017Salzburg 13-16 May 2017

Vilaca T, Walsh J, Eastell R

A meta-analysis of the risk of ankle and wrist fractures in diabetes - National Osteoporosis Society (NOS) Conference 2016 Birmingham 7-9 Nov 2016

Abbreviations

%CDL	Percent cortical distal load
%CPL	Percent cortical proximal load
%TDL	Percent trabecular distal load
%TPL	Percent trabecular proximal load
aBMD	Areal bone mineral density
AFF	Atypical femur fractures
AGE	Advanced glycation end products
BMD	Bone mineral density
BMSi	Bone material strength
BTM	Bone turnover markers
BV/TV	Bone volume/total volume
C.VM	Mean cortical Von Mises stress
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
Conn.D	Connectivity density
CRF	Clinical Research Facility
CSA	Cross sectional area
Ct.Ar	Cortical are
Ct.Pm	Cortical perimeter
Ct.Po	Cortical porosity
Ct.Po.Dm.SD	Cortical tissue mineral density
Ct.PoV	Cortical pore volume
Ct.Th	Cortical thickness
Ct.vBMD	Cortical volumetric bone mineral density
CTX	C-terminal cross-linked telopeptide of type 1 collagen
CV	Coefficient of variation
Dinn	Inner trabecular density
Dmeta	Meta trabecular density
DSPN	Distal symmetrical polyneuropathy
DXA	Dual energy X-ray absorptiometry
Ec.Pm	Endosteal perimeter
ESC	Electrochemical skin conductance
Est.Fail.Load	Estimated failure load
FESC	Feet electrochemical skin conductance
GLP-1	Glucagon like peptide 1
HESC	Hands electrochemical skin conductance
HR-pQCT	High resolution peripheral quantitative tomography
IGF-1	Insulin-like growth factor 1
ITS	Individual trabecula segmentation
LOX	Lysyl oxidase
µS	Microsiemens
MVD	Microvascular disease

NGH	Northern General Hospital
NOS	Newcastle- Ottawa Scale
NTX	N-terminal cross-linked telopeptide of type 1 collagen
NV	Non-vertebral
OC	osteocalcin
PINP	procollagen type I amino-terminal propeptide
PKC	Protein Kinase C
pQCT	Peripheral quantitative tomography
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analyses
Ps.Pm	Periosteal perimeter
PTH	Parathormone
QCT	Quantitative computed tomography
RHH	Royal Hallamshire Hospital
RPI	Reference point indentation
RRR	Ratio of relative risk
ScHARR	School of Health and Related Research
SD	Standard deviations
T1D	Type 1 diabetes
T1DN-	Type 1 diabetes without neuropathy
T1DN+	Type 1 diabetes with neuropathy
T2D	Type 2 diabetes
T2DFx	T2D group with fractures
Tb.Ar	Trabecular area
Tb.N	Trabecular number
Tb.Sp	Trabecular separation
Tb.TH	Trabecular thickness
Tb.vBMD	Trabecular volumetric bone mineral density
Tb.VM	Mean trabecular Von Mises stress
TCNS	Toronto Clinical Neuropathy Score
Tt.Ar	Total area
UK	United Kingdom
vBMD	Volumetric BMD
WHO	World Health Organization

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Chapter 1

Introduction

Chapter 1 Introduction

Introduction

Diabetes is a chronic disease characterized by hyperglycaemia. The incidence has been increasing worldwide for years and one in eleven adults has diabetes (1). According to the International Diabetes Federation Diabetes Atlas, 415 million people had diabetes in 2015 and a 5% increase in the number of patients is expected annually. Huge economic, social and medical burdens are associated with the disease (1).

Diabetes diagnosis

Diabetes diagnosis is based on hyperglycemia. According to the American Diabetes Association, diabetes diagnosis is based on a fasting blood glucose concentration above 7.0 mmol/L (126 mg/dL), a random blood glucose concentration above 11.1 mmol/L (200 mg/dL) with symptoms, or an abnormal result from an oral glucose tolerance test. In the absence of symptoms, the test should be repeated. Glycated hemoglobin can also be used, and concentrations above 48 mmol/mol (6.5%) are considered diabetes diagnosis. There are two main types of diabetes, type 1 and type 2 (2).

Type 1 diabetes

Type 1 diabetes (T1D) is a chronic disease characterized by autoimmune destruction of β-cells leading to insulin deficiency and hyperglycemia. The immunopathogenesis is described in Figure 1-1**Error! Reference source not found.** (3). In children, T1D onset commonly presents with polyuria, polydipsia and weight loss and approximately one third of the patients have diabetic ketoacidosis. Although juvenile onset is considered typical of T1D, people of any age can be affected and up to 50% of cases start in adulthood. Furthermore, adults might not present the classical symptoms and as many as 50% of the adults might be initially misclassified as type 2 diabetes (T2D) (3).

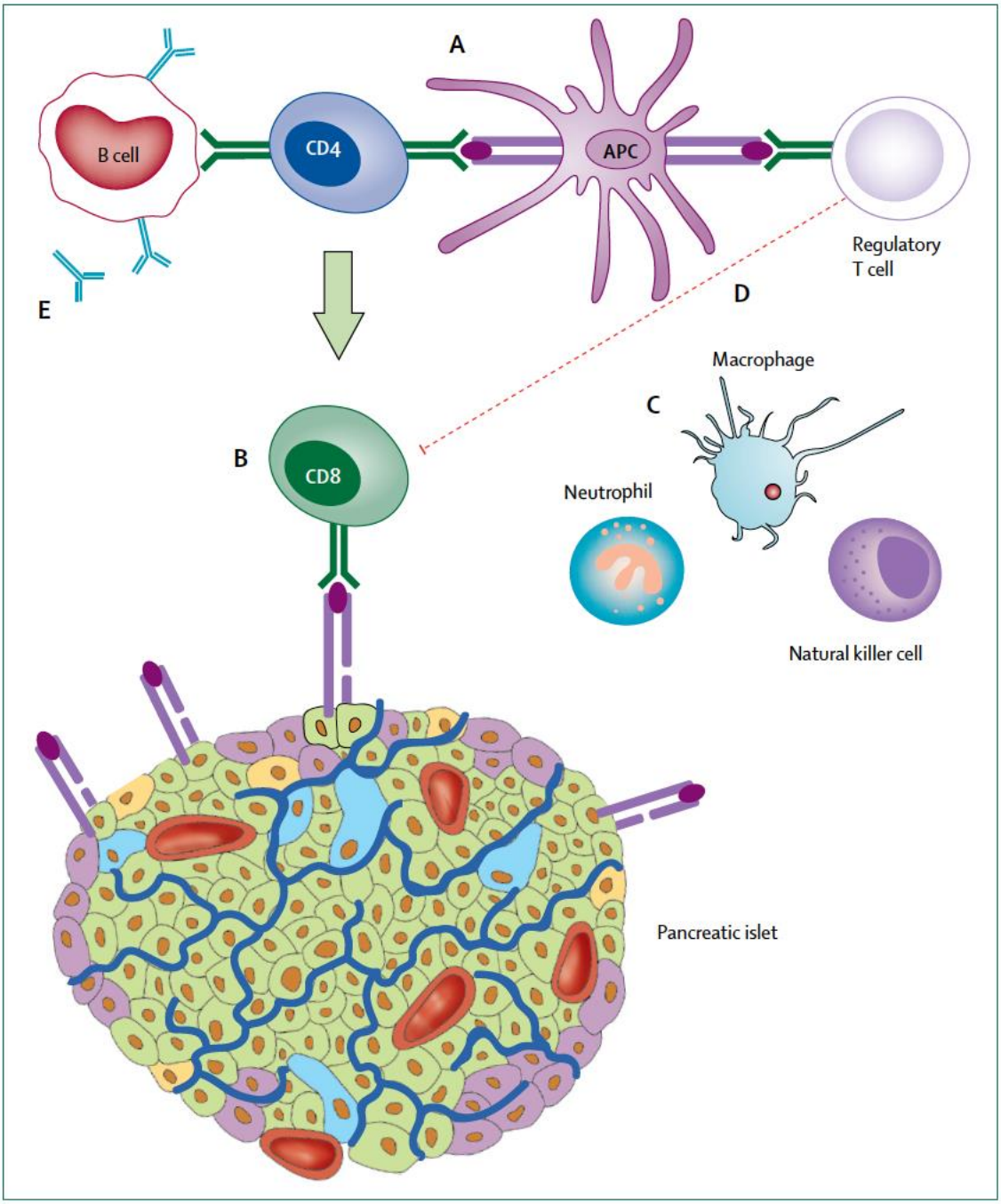

Figure 1-1The immunopathogenesis of type 1 diabetes

The development of type 1 diabetes is thought to be initiated by the presentation of β-cell peptides by antigen-presenting cell (APCs). APCs bearing these autoantigens migrate to the pancreatic lymph nodes where they interact with autoreactive CD4+ T lymphocytes, which in turn mediate the activation of autoreactive CD8+T cells (A). These activated CD8+ T cells return to the islet and lyse β cells expressing immunogenic self-antigens on major histocompatibility complex class I surface molecules (B). β-cell destruction is further exacerbated by the release of proinflammatory cytokines and reactive oxygen species from innate immune cells (macrophages, natural killer cells, and neutrophils; C). This entire process is amplified by defects in regulatory T lymphocytes, which do not effectively suppress autoimmunity (D). Activated T cells within the pancreatic lymph node also stimulate B lymphocytes to produce autoantibodies against β-cell proteins. These autoantibodies can be measured in circulation and are considered a defining biomarker of type 1 diabetes (E). In the pancreatic islet: green cells- beta cells; purple cells – alpha cells; blue cells- delta cells; red dots capillaries

Reprinted from The Lancet, (3) with permission from Elsevier.

T1D is a polygenic disease with sibling risk of 6-7% and offspring risk of 1-9%. The average lifetime risk is one in 250 people; however, it varies according to several factors such as geographic region and income. Both incidence and prevalence are increasing worldwide.
Currently, the peak incidence is in children aged 10-14 years but incidence is increasing particularly in children younger than 5 years old. Most people living with T1D are adults (3).

Type 2 diabetes

Conversely, pancreatic β-cell disfunction and insulin resistance in target organs leads to relative insulin deficiency in T2D. Frequently, the disease is asymptomatic. Recently, the combination of an ageing population, sedentary lifestyle and high obesity prevalence resulted in a substantial increase in the incidence and prevalence of T2D. More than 90% of patients with diabetes are affected by T2D (4).
In this population, the main cause of morbidity and mortality is cardiovascular disease. Glucose and lipid lowering strategies and blood pressure control reduces the risk of complications and cardiovascular disease progression (4).

Diabetes chronic complications

Despite diverse pathophysiology, hyperglycaemia is the common hallmark in T1D and T2D. Chronic hyperglycemia is the central initiating factor for microvascular disease (MVD). The common feature in the pathophysiology of MVD is a progressive narrowing of vascular lumen. Eventually this leads to occlusion and inadequate perfusion, hypoxia and impaired function. In addition, hyperglycemia activates several metabolic pathways. The four main pathways affected are; i) the polyol pathway; ii) the formation of advanced glycation end (AGE) products, iii) activation of protein kinase C; iv) hexosamine pathway flux.

The polyol pathway

The polyol pathway converts toxic aldehydes in inactive alcohols, through the action of the aldolase reductase enzyme. When glucose is high, aldolase reductase converts glucose to sorbitol. The process consumes NADPH, an important cofactor in the regeneration of glutathione, a critical antioxidant. This reduction of intracellular antioxidant increases susceptibility to intracellular oxidative stress (Figure 1-2) (5).

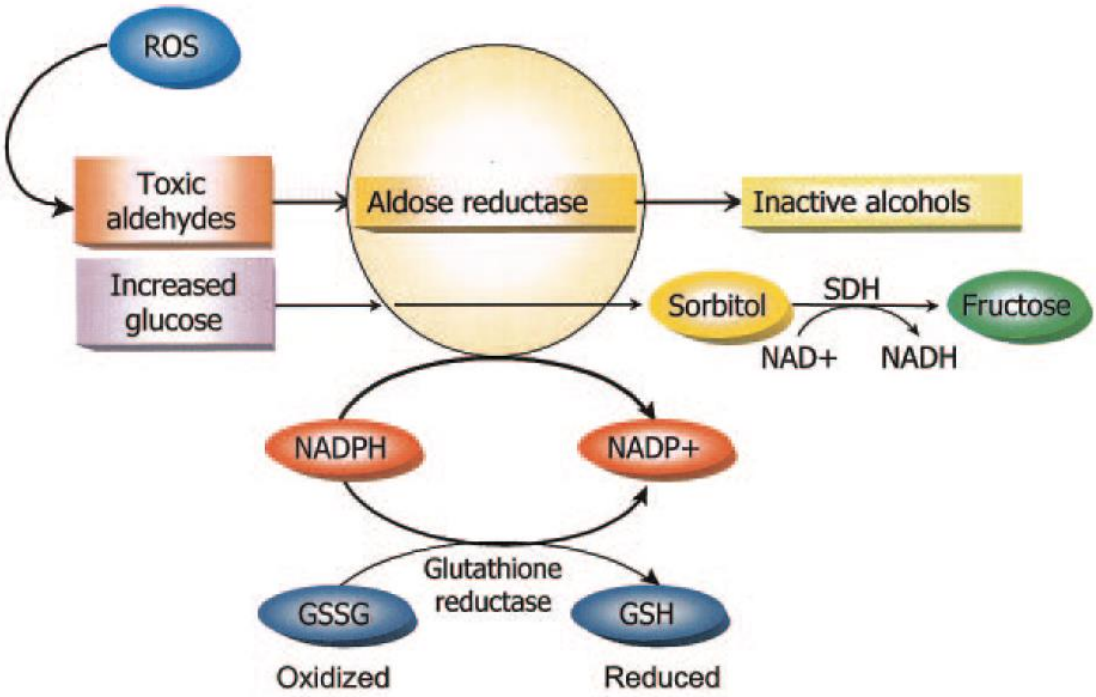

Figure 1-2Hyperglycemia increases flux through the polyol pathway

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The production of AGEs

Glucose and other sugars can react with proteins producing AGEs. This post-translational modification might influence the protein function. Intracellularly, this could influence several interactions, including the regulation of gene transcription; on the extracellular matrix, this could modify the interaction with extracellular matrix nearby. Finally, modified proteins might circulate in the blood stream. There are receptors for AGE and the activation of this receptors is associated with inflammation (Figure 1-3) (5).

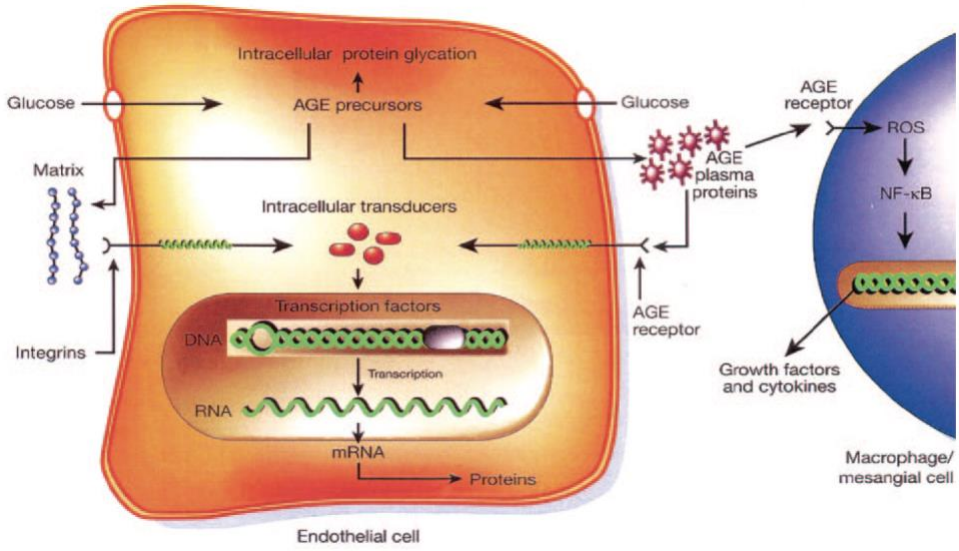

Figure 1-3 Increased production of AGE precursors and its pathologic consequences.

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Protein kinase C activation

Protein Kinase C (PKC) is a multifunctional protein involved in a variety of important cellular pathways. Diacylglycerol is a critical activation cofactor of many isoforms of PKC. Hyperglycemia increases the synthesis of diacylglycerol, leading to several effects on gene expression (Figure 1-4) (5).

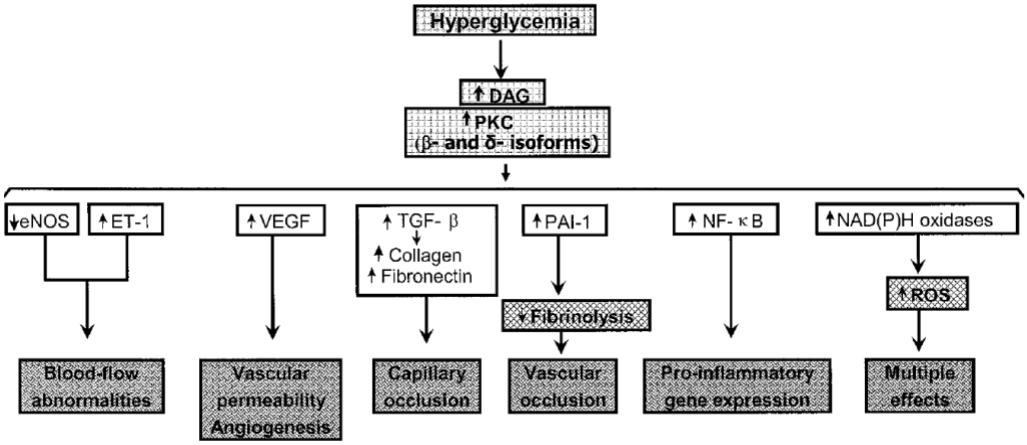

Figure 1-4 Consequences of hyperglycemia-induced activation of PKC.

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Increase hexosamine pathway activity

In hyperglycemia, most of the glucose is metabolized thorough glycolysis. The excess of glycolysis generates products that might react with transcription factors and result in pathologic changes in gene expression (Figure 1-5) (5).

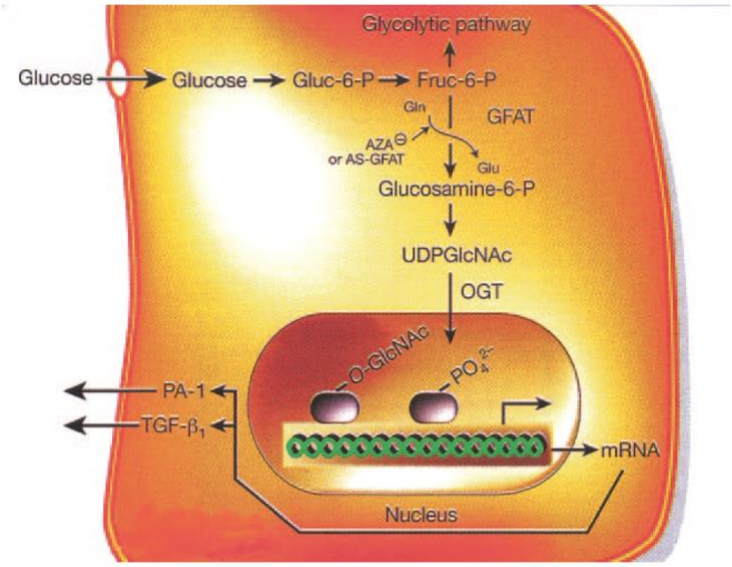

Figure 1-5 Hyperglycemia increases flux through the hexosamine pathway

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Although these pathways are not directly linked their activation eventually results in increase in oxidative stress (Figure 1-6) (5).

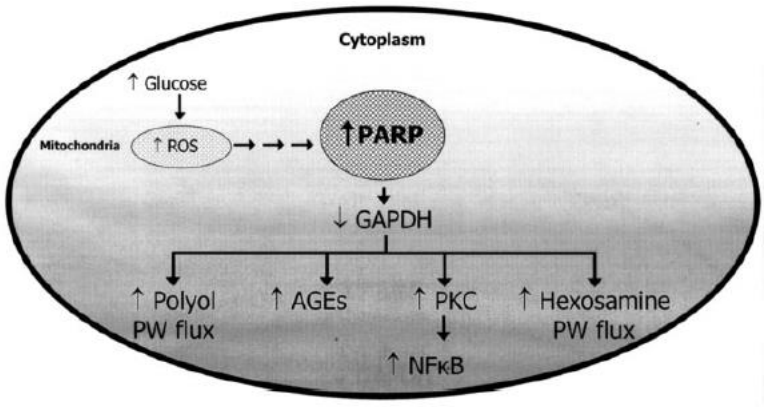

Figure 1-6 The unifying mechanism of hyperglycemia induced cellular damage.

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Although hyperglycemia is a systemic phenomenon in diabetes, microvascular complications are restricted to specific sites; retinopathy affects the retina, nephropathy affects the kidneys and neuropathy affects the nerves. The reason for this finding is that most cells control glucose transport and maintain intracellular glucose concentrations constant despite extracellular hyperglycemia. However, endothelial and mesangial cells at the retina, glomerulus and nerves do not control glucose influx and develop intracellular hyperglycemia (5).

Since patients with neuropathy were involved in the clinical study, neuropathy will be discussed in further details.

Neuropathy

Definition and symptoms

Diabetic neuropathy is a result of nerve damage and typically presents as sensory abnormalities. The Toronto Consensus Panel on Diabetic Neuropathy defined Distal symmetrical polyneuropathy (DSPN) as 'a symmetrical, length dependent sensorimotor polyneuropathy attributable to metabolic and microvessel alterations as a result of chronic hyperglycaemia exposure (diabetes) and cardiovascular risk covariates' (6). Neuropathy can cause negative symptoms such as impaired touch, vibration, pinprick, hot and cold sensation or positive symptoms such as paradoxical pain and hypersensitivity. Symptoms are typically distal, with the classical stocking–glove pattern (Figure 1-7). DSPN is the most common form but other manifestations include mononeuropathy (while affecting single nerves), plexopathy (while affecting a nerve plexus), radiculopathy (while affecting nerve roots) diabetic autonomic neuropathy (while affecting the autonomic nervous system) (Figure 1-7) (7).

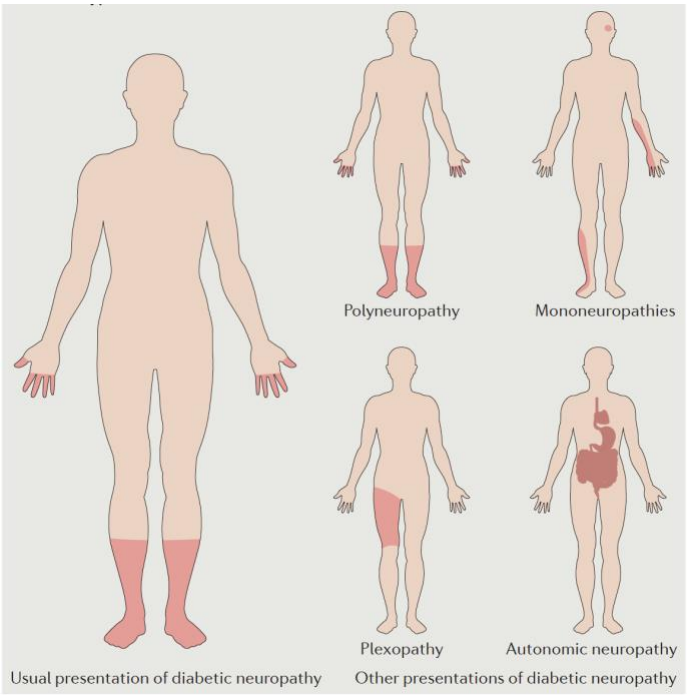

Figure 1-7 Diabetic neuropathy presentations

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Epidemiology

Diabetic neuropathy is a common complication of diabetes. In the Rochester Diabetic Neuropathy cohort, a neuropathic disorder was reported in 59% of T1D patients and 66% of T2D (8). The most prevalent manifestation is DSPN, present in around 50% of patients with diabetes (8). Around a fifth of patients with diabetes develop DSPN. Burning, 'electric-shock type' and sharp pain are the most common symptoms, but aching, itching, cold pain and others are often described. Symptoms are often worst at night. Most of the patients grade the pain as severe. Quality of life is often impaired (9).

Mechanisms

Several vascular and metabolic mechanisms are associated with the impairment of nerve function in diabetes (5, 7, 9). As previously discussed for MVD, the several metabolic pathways activated and the increased oxidative stress leads to abnormal nerve function and nerve injury (Figure 1-8).

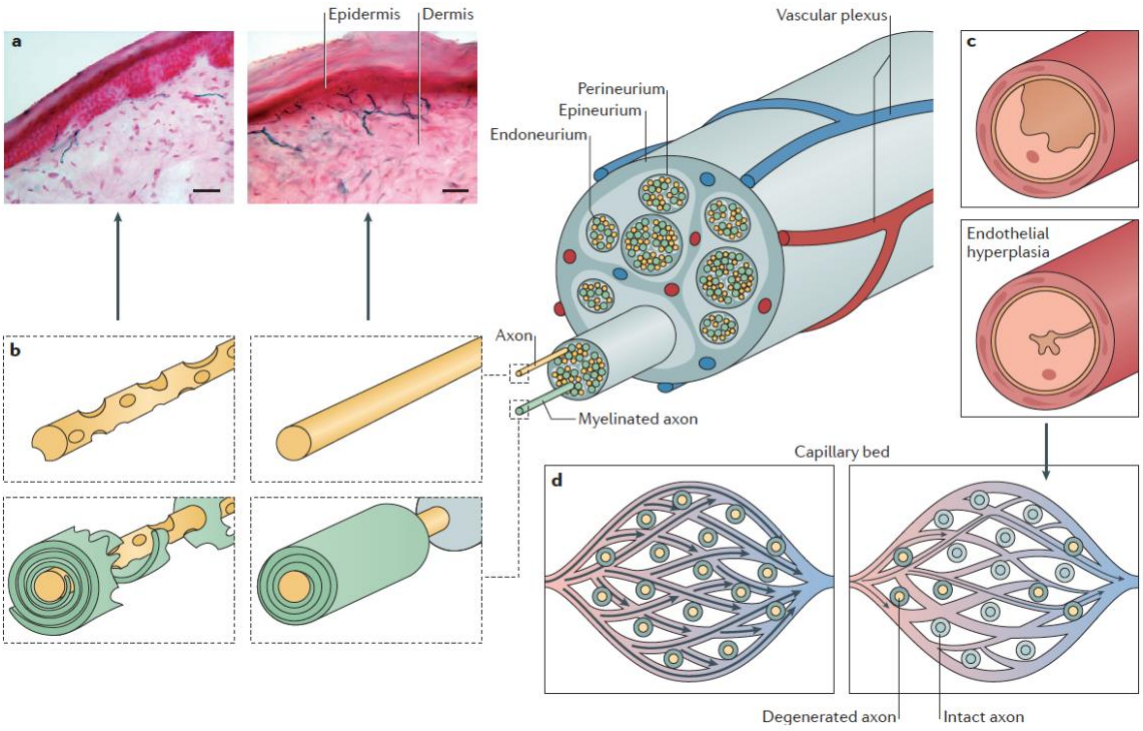


Figure 1-8 Pathogenesis of diabetic neuropathy

Diabetic neuropathy has a complex pathogenesis involving interaction of axonopathy, schwannopathy and microvasculopathy. The figure shows the anatomical organization of myelinated and unmyelinated axons within nerve fascicles. Their nutrient supply is secured via endoneurial capillaries which, together with the perineurial membrane, form the blood–nerve barrier. ***a*** *| Human skin biopsy samples immunostained with PGP9.5 to show progression of peripheral nerves from the dermis into the epidermis, where they exist as small unmyelinated C-fibres (scale bar 40 µm). Left panel shows loss of fibres in a patient with diabetic neuropathy and right panel shows fibres in a healthy individual.* ***b*** *| Changes in axons and myelin in diabetic neuropathy, showing degeneration of Schwann cells and nerve fibres, culminating in nerve and intraepidermal fibre loss.* ***c*** *| Endoneurial capillaries from patients with diabetes. Top panel shows a capillary from a patient without diabetic neuropathy, and bottom panel shows a capillary from a patient with neuropathy, in which endothelial cell hyperplasia and basement membrane thickening have reduced the size of the capillary lumen.* ***d*** *| Narrowing of individual capillaries might not prevent blood from passing through the endoneurial capillary bed per se, but the resulting increase in velocity of blood through endoneurial functional shunts or epineurial arteriovenous shunts prevents efficient oxygen extraction, causing hypoxia. Reprinted from Nature reviews (7) with permission from Elsevier.*

Besides the four main pathways reported, other mechanisms might also be involved as myoinositol and neurotrophin depletion, reduced Na^+, K^+, ATPase activity, schwannopathy, mitochondrial disfunction and increased inflammatory response (Figure 1-9) (7). Despite several advances, the mechanisms that leads to diabetes neuropathy are not fully understood.

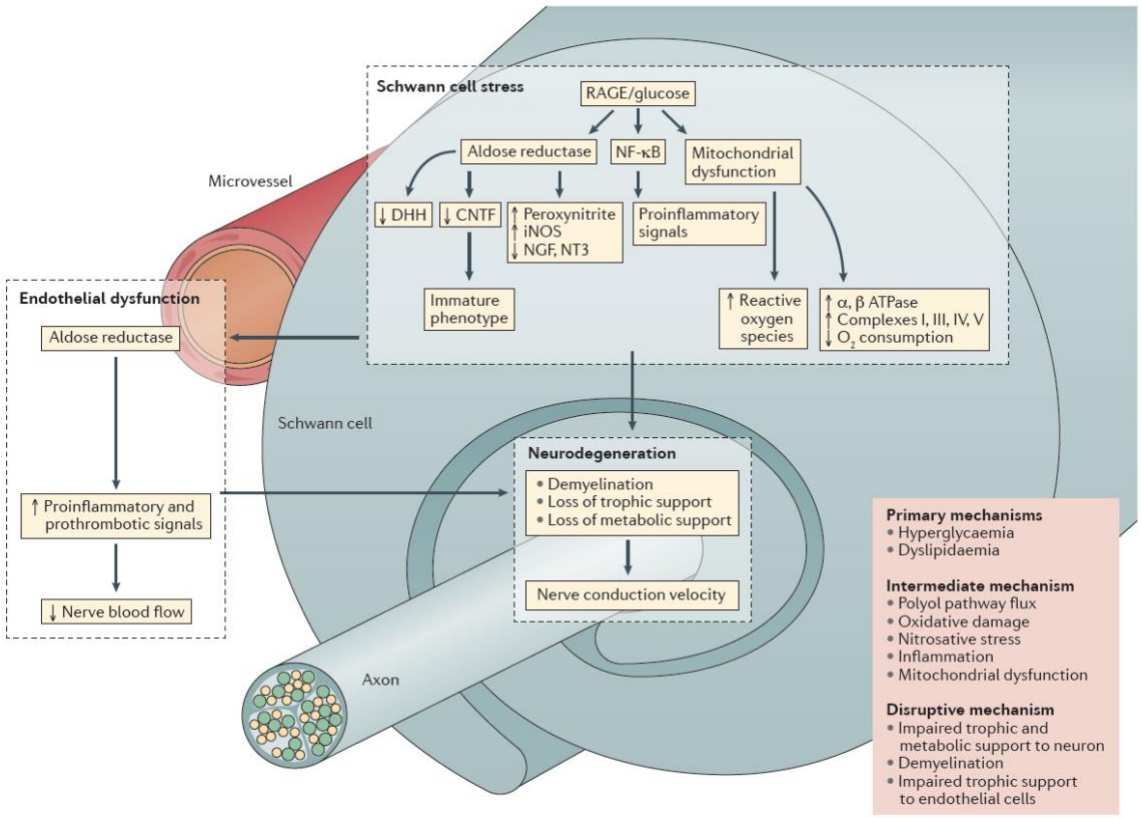


Figure 1-9 Hyperglycaemia-driven Schwann cell stress and neurodegeneration.

Hyperglycaemia and dyslipidaemia ultimately lead to reduction of neuronal support from Schwann cells and microvessels. In Schwann cells, RAGE (receptor for advanced glycosylation end products) signalling leads to increased glucose metabolism by aldose reductase, which generates local oxidative damage, causes inflammation and drives cells to an immature phenotype. It also affects mitochondrial function, which increases oxygen consumption, and reduces production of desert hedgehog (DHH), which affects endothelial cell function. Endothelial cells also express aldose reductase, and increased polyol pathway flux activates proinflammatory and prothrombotic pathways that reduce nerve blood flow. Disruption of neuronal support by Schwann cells and the vascular system contributes to neuropathy, in conjunction with the direct effects of diabetes on neurons themselves. Reprinted from Nature reviews (7) with permission from Elsevier.

Risk of fractures in diabetes

A number of meta-analyses have reported an increased risk of fractures in people with diabetes (10-14). Janghorbani et al (10) and Vestergaard (11) published the first meta-analyses in 2007, and since then, other authors have observed similar findings (Table 1.1, Table 1.2).

Table 1.1 Risk of hip and spine fractures inT1D and T2D according to meta-analyses

Study, Year	N	Age range	Hip fractures		Spine fractures	
			T1D	T2D	T1D	T2D
Janghorbani, 2007	836,941	all ages	6.3 (2.6 - 15.1)*	1.7 (1.3 - 2.2)*	-	1.2 (0.7 – 2.2)
Vestergaard, 2007	NR	NR (means 43-73.5)	6.94 (3.2–14.78)*	1.38 (1.25–1.53)*	-	0.93 (0.63–1.37)
Fan, 2015	6,995,272	20-97	5.76 (3.66 – 9.07)*	1.34 (1.19-1.51)*	-	-
Dytfeld, 2016	765,121	> 50	-	1.26 (1.07 – 1.57)*	-	1.13 (0.94-1.37)
Shah, 2015	4,391,425	> 20	3.78 (2.05-6.98)*	-	2.88 (1.71-4.82)*	-
Wang, 2018	NR	20-84	4.35 (2.91-6.49)*	1.27 (1.16-1.39)*		1.74 (0.96-3.26)

* statistically significant; N (number of participants) and age range for the whole analysis; the authors did not report individual data for each analysis.

The risk varies according to the type of the disease and the skeletal site. The highest risk was found at the hip ranging from 3 to 7 fold in T1D (10-12, 14) and from 26 to 70% T2D (10, 11, 13, 14). For vertebral fractures, no increase in risk was found in T2D (10, 11, 13) and Shah et al described an increased risk in T1D (14) . In the 'any fracture risk' analysis, a greater risk was observed in T1D (Table 1.2) (14).

Table 1.2 Risk of any fractures in T1D and T2D according to meta-analyses

Study	Any fractures		N participants	Age range (y)
	T1D	T2D		

Janghorbani (10)	-	1.2 (1.01-1.5)*	836,941	all ages
Vestergaard (11)	-	1.19 (1.11-1.27) *	NR	NR (means 43-73.5)
Shah(14)	3.16 (1.51-6.63)*	-	4,391,425	> 20
Wang	1.51 (1.35-1.68)*	1.22 (1.13 to 1.31)	NR	20-84

* statistically significant N (number of participants) and age range for the whole analysis; the authors did not report individual data for each analysis.

Table 1.3 Fracture risk in T2D according to Janghorbani et al

Site	RR	95% CI
Hip*	1.7	1.3 – 2.2
Any fractures*	1.2	1.01 – 1.5
Foot *	1.3	1.1 – 1.7
Distal forearm	0.98	0.8 - 1.2
Ankle	1.3	0.9 – 2.0
Proximal Humerus	1.3	0.8 – 2.2
Vertebra	1.2	0.7 – 1.2

* Statistically significant

Janghorbani *et al* evaluated other skeletal sites in T2D, such as the distal forearm, ankle, proximal humerus and foot and only in the last one a significant increase in the risk was found (Table 1.3) (10).

A large study from the Scottish National Registry showed interesting data. More than 3.86 million people were evaluated, 3.66 million non-diabetic controls, 21,033 T1D and 180,841 T2D (15). There was an increase in the risk of hip fracture in T1D (RR 3.28 95% CI 2.52-4.26 in men and 3.54 95% CI 2.75-4.57 in women) but in T2D there was no increased risk in men (RR 0.97 95% CI 0.92-1.02) and a small increase in the risk in women (RR 1.05 95% CI 1.01-1.10). Nevertheless, the risk was increased in T2D with more than 7 years of disease duration in both genders (RR 1.25 95% CI 1.08-1.45 for men and RR 1.55 95% CI 1.38-1.75 for women) (15). This data suggests that bone could be a target to diabetes complications, clinically manifested in a subset of patients with longer disease length.

Bone quantitative analysis in diabetes

Bone mineral density

Bone mineral density (BMD) is one of the main tools used to evaluate the risk of fractures in clinical practice. Estimates suggest that 70% of bone strength could be attributed to BMD (16). Usually, an inverse relationship is observed between BMD and the risk of fractures, however in the diabetic population, the findings do not follow the expected pattern. In T1D patients, BMD is decreased. Vestergaard described a decrease in BMD Z-score in spine (mean ±SEM -0.22±0.01) and hip (-0.37±0.16) (11) and Pan et al, also in a meta-analysis, described a more comprehensive evaluation (Table 1.4) (17).

Table 1.4 Pooled mean difference (MD) of BMD between T1D and non-diabetic individuals according to meta-analysis by Pan

Bone sites	MD (g/cm^2)	95% CI	p
Total body	-0.06	-0.1, -0.01	0.013
Spine	-0.03	-0.08, 0.02	0.238
Spine female	-0.01	-0.04, 0.01	0.327
Spine males	-0.04	-0.07, -0.01	0.003
Femur	-0.06	-0.13, 0.00	0.049
Femur <20y	-0.04	-0.05, -0.03	<0.001
Hip female>20y	-0.05	-0.08, -0.02	0.001
Hip male > 20y	-0.02	-0.06, 0.02	0.001
Forearm fem>20y	-0.01	-0.02, 0.00	0.023
Forearm male>20y	0.00	-0.02, 0.02	0.777

MD: mean difference; CI: confidence interval; y: years; fem: female
Adapted from ref (17).

A number of features could contribute to the decrease in BMD in diabetes. In early-onset T1D, hyperglycaemia and poor metabolic control may compromise growth hormone and insulin-like growth factor 1 (IGF-1) actions in bone modelling (18), leading to a suboptimal peak of bone mass accrual (11). Furthermore, the lack of insulin's anabolic effect and the glucose toxicity may also affect bone remodelling and result in a decrease in BMD even in adults (11, 19). It would be expected that this decrease in BMD would result in an increase in the risk of fractures, however, the increase in the risk of fractures observed, for example, at the hip (RR 6.94) is much higher than would be expected (RR 1.42) for the given BMD decrease (11). Therefore, BMD should be interpreted with caution in diabetes. Schwartz *et al* evaluated the association between BMD and the risk of fractures in T2D (20). In this study, femoral BMD

was associated with the risk of fractures, however, for a given T-score and age, people with T2D have a greater risk of fractures than people without diabetes. People with T2D have fractures in a higher BMD than general population, suggesting that BMD cannot capture the abnormalities in diabetes.

Computed tomography

Bone involvement in T1D was also evaluated by computed tomography. Ishikawa et al assessed femoral and spine structure using quantitative computed tomography (QCT) in 17 male T1D diabetics with a mean duration of the disease of 15.6 (±8.6) years, mean haemoglobin A1c 7.4% ±0.9 (ranging from 6.4 – 10.3%) and 88% of them without retinopathy. A significantly lower cortical volumetric BMD (vBMD) in the femoral neck and vBMD cortical thickness and cortical cross sectional area (CSA) in the intertrochanter were described, but no difference was detected at the spine (21). T1D adolescents were evaluated using peripheral quantitative tomography (pQCT) and reduced bone mineral content and smaller bone CSA were detected (22). Therefore, a reduction in CSA (pQCT) has been reported in adolescents and in cortical CSA (QCT) in adults without MVD.

In T2D, the evaluation of bone structure by quantitative computed tomography showed greater hip areal bone mineral density (aBMD) associated with greater trabecular vBMD (23). Cross sectional area, cortical thickness and cortical vBMD were similar between T2D group (n=49) and healthy controls. There were no data in regard to the length of the disease or metabolic control in participants with diabetes (23). The data are in accordance with densitometry measurements suggesting that bone quality and not quantity is affected in T2D.

Bone quality in diabetes

Microarchitecture

T1D

High resolution peripheral quantitative tomography (HR-pQCT) evaluates vBMD and bone structure at the radius and tibia *in vivo*. The 82 μm resolution enables this tool to assess structural properties of bone near to a trabecular level (Figure 1-10) (24). The device was used to evaluate adult patients with diabetes with and without MVD (25). Summary of HR-pQCT findings in diabetes is described in Table 1.5. In T1D, the group with MVD exhibited lower total, trabecular and cortical vBMD and thinner cortex at the radius and lower total and

trabecular vBMD at the tibia, compared with healthy controls. No difference was reported between diabetics without MVD and healthy controls. In regard to cortical porosity there was no difference between any of the groups.

Table 1.5 Summary of HR-pQCT findings in T1D and T2D

Study	Group/ site assessed (n)	Trabecular findings		Cortical findings		Finite element analysis	
		Radius	Tibia	Radius	Tibia	Radius	Tibia
Shanbhogue (25) [a]	T1D MVD + (n=26) x healthy controls (n=26)	↓ vBMD	↓ vBMD	↓ vBMD ↓ cortical thickness			
	T1D MVD+ (26) x T1D MVD – (29)	↓ total vBMD (11%) ↓ Tb vBMD (18%) ↓ Tr.Th (12%)	↓ total vBMD (17%) ↓ Tb vBMD (20%) ↓ Tb.Th (14%), ↑Tb.Sp (11%) ↑ Tb inhomogeneity (16%)		↓ tibial cortical area (25%)	↓ total bone stiffness (14%,) ↓ failure load: (14%)	↓ bone stiffness (16%) ↓failure load (15%)
Burghardt (26)	T2D (n=19) x healthy controls (n=19)		↑ vBMD adjacent to the cortex ↑ Tb.Th	↑ cortical porosity			
Paccou (27)	T2D (M=18; W=11) x healthy controls (M177; W155)			↑pore volume (W)	↑ cortical porosity (M) ↑ pore volume (M)		
Shanbhogue (28) [b]	T2D MVD+ (n=25) x			↓ vBMD ↓ cortical thickness			

Study	Group/ site assessed (n)	Trabecular findings		Cortical findings		Finite element analysis	
	healthy controls (n=26)			↑ cortical porosity			
Patsch (29) [c]	Distal scanning (24.5 mm from reference line at radius and 37.5mm at the tibia) T2D with fracture (n=20) x T2D without fracture (n=20)	↑ trabecular heterogeneity		↑ relative porosity ↑ pore volume	↓ vBMD ↑ pore volume ↑ porosity ↑ endocortical bone surface		
Nilsson	T2D (n=99) Healthy controls (n=954) Standard Analysis	↑ BV/TV (+15%)	↑ BV/TV (+11%)				
	T2D (n=99) Healthy controls (n=954) Distal (14% length)			↑ vBMD (1.7%), ↑ cortical area (9.3%) ↓ cortical porosity	↑ vBMD (1.6%) ↑ cortical area (11.5%)	↑ failure load (12.9%)	↑ failure load (7.7%)
Samelson	T2D (n=129) Controls (n=940)				↓ cortical vBMD ↑ cortical porosity		

Study	Group/ site assessed (n)	Trabecular findings		Cortical findings		Finite element analysis	
	(40-87 y; mean 64± 8)				↓ CSA		
	T2D with (n=42) vs T2D without fractures (n=87)			↓ cortical thickness ↓ cortical porosity	↓ cortical vBMD, ↓ tissue mineral density	↓ failure load	
	Controls fractures (307) vs no fracture (n=633)	↓ Tr vBMD, ↓ Tr.N, ↑ Tr.Sp, ↑ Tr.Sp SD; ↓ Tr.Th	↓ Tr vBMD, ↓ trabecular number, ↑ trabecular separation, ↑ trabecular separation SD			↓ failure load	↓ failure load
Starr (individual trabecular segmentation)	T2D (n=42) vs controls (n=50)	ITS greater plate-like and less rod-like trabecular network (DM< 10 years)					
Farr	T2D (n=30) vs non-T2D (n=30) post-menopausal women (age matched)	no difference after adjustment for covariates		no difference after adjustment for covariates			

[a] no difference was found between T1D without MVD and controls

[b] no difference was found between T2D without MVD and controls
[c] no significant difference was found between T2D without fractures and controls; there was a trend to lower cortical pore volume and lower relative cortical porosity in distal radius in T2D without fractures;

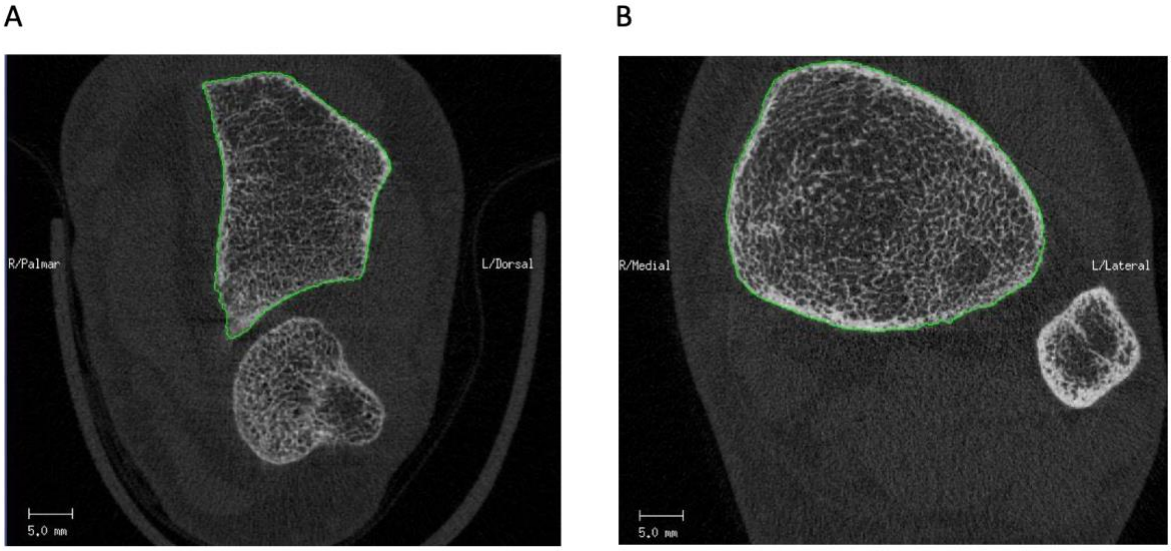


Figure 1-10 HR- pQCT bone images. A – radius; B tibia;

T2D trabecular compartment

Several studies reported HR-pQCT data in T2D and results were inconsistent. In the trabecular compartment, Burghardt et al reported increased vBMD adjacent to the cortex and trabecular thickness at the tibia. There was no difference in other trabecular features at the tibia or radius (26). Individual trabecula segmentation (ITS) analysis characterises the morphology of trabecular bone. ITS analysis comparing T2D and controls without diabetes reported greater plate-like and less rod-like trabecular network in early T2D (less than 10 years duration) but not in people with longer diabetes duration (30). Furthermore, a Swedish study assessed 75-80-year-old women, one quarter of them with newly diagnosed T2D, reported greater BV/TV at both the tibia and radius. No other study reported significant findings in the trabecular compartment in T2D (31).

T2D cortical compartment

In the cortical compartment, the most common finding was on cortical porosity. Five studies compared T2D and healthy controls with four reporting an increase in cortical porosity; three of them at the radius (26, 28, 32) and one at the tibia (27). Conversely, the Swedish study reported a decrease in cortical porosity at the radius. This finding came from a non-standard site (at 14% of the bone length, less distal than the standard site), a site rich in cortical bone. No difference in cortical porosity was found in the standard analysis (31). Conflicting results were also found while comparing T2D with and without fractures. Patsch et al analysed the

standard site but also a less distal site, at 22.5 mm from the reference line at the radius and 37.5mm at the tibia. At the less distal site, an increase in cortical porosity at the radius was found in the T2D group with fractures (T2DFx) compared to T2D without fractures (29). Conversely, Samelson reported a decrease in cortical porosity at the radius at the standard site in T2DFx, compared to T2D. Interestingly, in the study by Samelson, other cortical indices were worse in the T2D group with fractures (lower tibia cortical vBMD, lower tibia cortical tissue mineral density and lower radius cortical thickness) (32).

Bone strength in diabetes

Conflicting results were also found in the bone strength analysis. In accordance with the favorable microarchitectural findings, failure load and stiffness calculated by finite element analysis were higher at the radius and tibia in the proximal analysis reported by Nilsson (comparing T2D and controls) (31). In the other studies comparing T2D or T1D and controls there were no differences in bone strength assessed by finite element analysis.

Samelson et al, while comparing individuals with and without fractures, found lower failure load at the radius both in T2D (T2D with fractures vs T2D without fractures) and controls (controls with fractures and controls without fractures) and also lower failure load at the tibia in controls with fractures compared to controls without fractures (32).

Reference point indentation

Reference point indentation (RPI) is also used to evaluate the toughness of the bone. It measures the distance that a probe (Osteoprobe ®) descends into the bone for a given applied force (33). The greater the distance, the lower is the bone material strength (BMSi). In postmenopausal women with diabetes (n=19) BMSi was 9.2% lower than in the controls and was inversely associated with the duration of the disease (33).

Two studies have assessed both microarchitecture and BMSi. Farr et compared 30 postmenopausal women with T2D >10 years and 30 non-diabetic age-matched controls. Microarchitecture was not different between the groups but BMSi was around 10% lower in the group with diabetes. In T2D patients, BMS correlated negatively with the average HbA1c levels in the last 10 years (34).

Furthermore, Nilsson et al, in the Swedish study also reported lower BMSi in T2D compared to controls despite lower cortical porosity (31). BMSi is suggested to be an *in vivo* measure of

bone material properties. These findings suggest that impairment of bone matrix properties could contribute to bone fragility in diabetes.

Histomorphometry in diabetes

There are not many studies evaluating bone histomorphometry in people with diabetes. Armas et al found no differences in structural parameters when comparing T1D (n=29) without MVD and healthy controls (Figure 1-11) (35). While evaluating a subset of patients with previous fragility fractures, subtle differences in structure towards lower bone remodelling parameters (non-significant lower bone volume/ total volume- BV/TV and trabecular thickness TbTh) and mineralization (significantly shorter mineralization lag time) were observed (36, 37), suggesting an impairment in bone formation in this group.

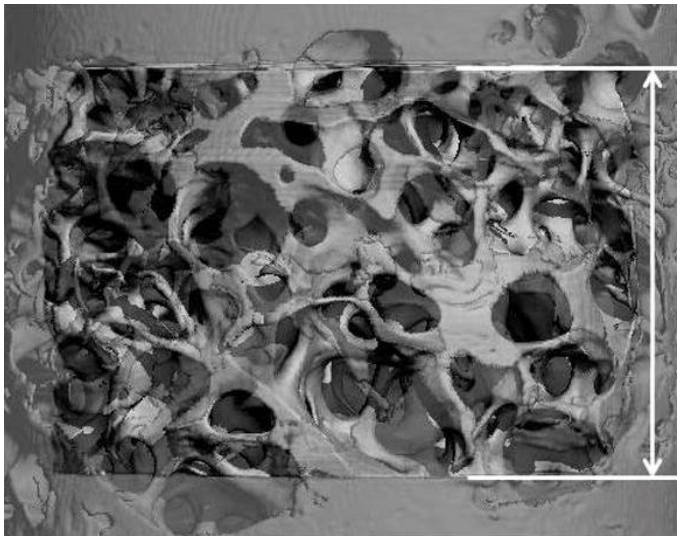


Figure 1-11 The 3D- image of bone biopsy. Trabecular bone.

In T2D, decreased bone and osteoid volume, decreased osteoid thickness and lower osteoblast surface were observed in post-mortem iliac crest samples of T2D (n=26) compared to controls (38). These findings were considered suggestive of decreased bone formation (38). In a comparison of iliac crest samples between T2D (n=5) and healthy controls (n=4), no differences in trabecular parameters were found, but cortical width and area were significantly decreased in people with diabetes (39). In regard to dynamic indices, mineralizing

surface, bone formation rate, osteoid and osteoblast surfaces presented significantly lower values (*Table 1.6*) (39).

Table 1.6 Histomorphometric indices in T2D and healthy controls in trans iliac biopsies (39)

Histomorphometric indices	DM2 (n=5)	Healthy controls (n=4)	p
Mineralizing surface (%)	2.65 ±1.9	7.58 ± 2.4	0.02
Bone formation rate ($\mu m^3/um^2.d$)	0.01 ±0.1	0.5 ± 0.2	0.02
Osteoblast surface (%)	1.23 ± 0.9	4.6 ± 2.5	0.03

Recently, Andrade et al compared bone biopsies of 26 T2D premenopausal women with 15 age, sex and race matched controls. Bone volume was greater in T2D compared to controls. Within the T2D group, the effect of metabolic control and MVD (retinopathy and nephropathy) was assessed. Poor metabolic control showed a decrease in static parameters of bone formation when compared to good metabolic control. Static parameters correlated negatively with HbA1c levels. MVD was associated with reduction in static (osteoid thickness and osteoid surface) and dynamic parameters (mineralizing surface, bone formation rate, and mineral apposition rate) of bone formation and mineralization. These findings suggested a decrease in bone turnover in T2D, associated with poor metabolic control. MVD exacerbated the findings (40).

Bone turnover markers (BTM) in diabetes

Several studies reported a decrease in bone turnover markers in diabetes (25, 28, 29, 41-43). Shanbhogue et al reported lower levels of C-terminal cross-linked telopeptide of type 1 collagen (CTX), osteocalcin (OC) and procollagen type I amino-terminal propeptide (PINP) in T1D (25) and T2D(28). A meta-analysis described a significant decrease in OC and CTX in diabetic patients and no difference in the other BTM and calciotropic hormones (alkaline phosphatase, N-terminal cross-linked telopeptide of type 1 collagen (NTX), parathormone (PTH), 25-hydroxy-vitamin D (42). A direct influence of glucose on the measurements was excluded by a methodological study that evaluated the effect of adding glucose to fasting samples (42). Although there are some discordant results, in general, BTM are lower in diabetic patients, suggesting a decrease in bone turnover.

Advanced glycation end products

Type 1 collagen is the main component of the organic bone matrix. It is composed by three chains in a helical conformation. Enzymatic hydroxylation promoted by the lysyl oxidase (LOX) establish intra and intermolecular crosslinks that confers stiffness to the structure (Figure 1-12) (44).

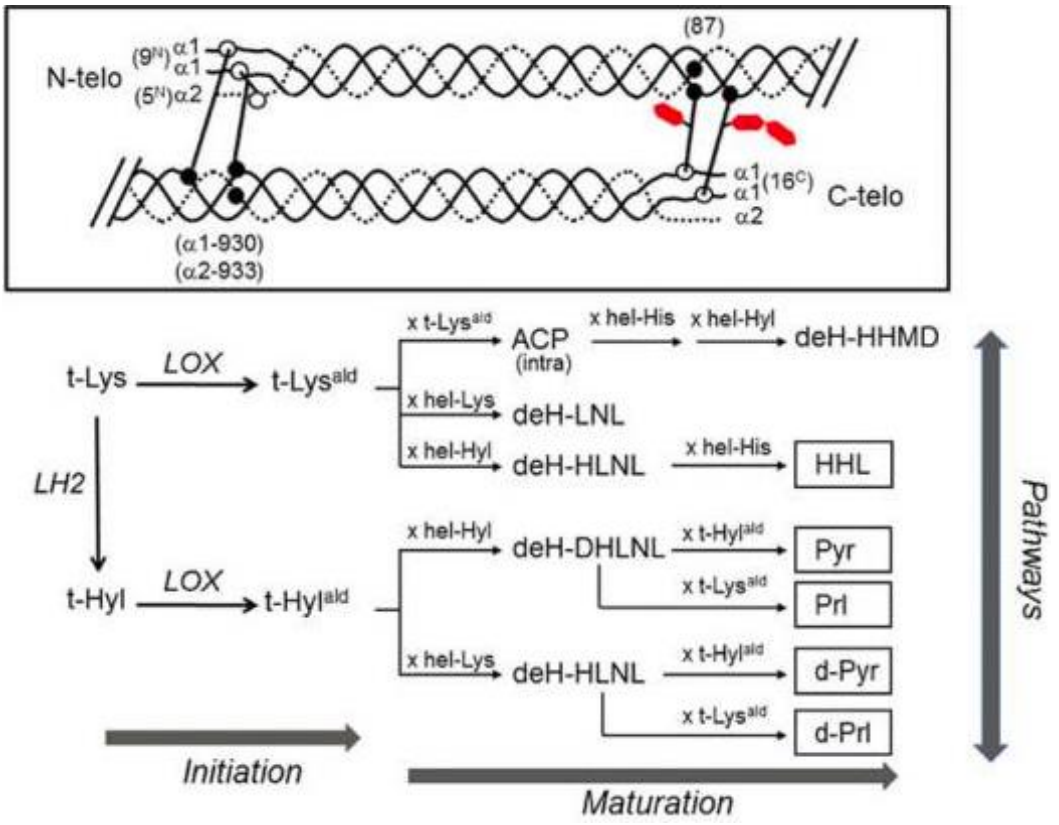


Figure 1-12 LOX mediated collagen cross-linking

Since early embryonic life non-enzymatic post-translational reactions occur in several molecules, including collagen and they continue throughout lifetime. The accumulation of these reactions has been associated with normal ageing, pathologic processes and neurodegenerative diseases (45). The addition of sugar residues, called glycation, is one of these post-translational modifications. In a diabetic environment, the glucose excess can promote glycation of residues leading to the formation of AGEs (45). They include crosslinking modifications within or across collagen fibres such as pentosidine, vesperlysines and

crosslinking and non-crosslinking modifications such as carboxymethyllysine, carboxyetillysine and pyrraline (46). The low turnover of collagen fibres favours the accumulation of these products and can result in modifications of physical properties of the fibres such as stiffness and enzyme resistance.

The charge profile of the molecule can also be modified, affecting the interaction within the fibres and between fibres and cells, which could affect repair of tissue damage (46). It has been demonstrated that AGEs influence normal osteoblast development and function like attachment to the collagen matrix (47). Analyses of human bone *in vitro* showed that cancellous bone is more prone to the formation and accumulation of the glycation products (45).

Pentosidine is the best characterized AGE in the bone field. The molecule is formed by reactions involving pentoses that are not the main sugar in human metabolism. Therefore, pentosidine accounts for less than 1% of AGEs in bone (Figure 1-13) (45). However, the molecule can be measured by immunoassays in blood and urine. Furthermore, pentosidine is fluorescent and fluorescence can be measured in tissue samples *in vivo*. These characteristics make the molecule an accessible AGE (34). The evaluation of cadaveric vertebrae has reported a negative relation between bone pentosidine levels and mechanical properties (16). In a 5-year prospective study, urinary levels of pentosidine have been described as an independent risk factor for osteoporotic vertebral fractures in non-diabetic women (48). Higher urinary pentosidine levels were also considered a risk factor for fractures in T2D men and women aged 70-79 years (49). In addition, serum pentosidine levels were significantly higher in T1D with previous fractures and in multivariate logistic regression, pentosidine was considered an independent factor associated with prevalent fractures (50). More recently, Farlay et al evaluated bone histomorphometry and pentosidine content in iliac crest samples in T1D with and without fractures and healthy controls. Although the sample was small (n=5 in each group) a significantly higher content of pentosidine was reported in T1D with fractures compared to the healthy controls (37).

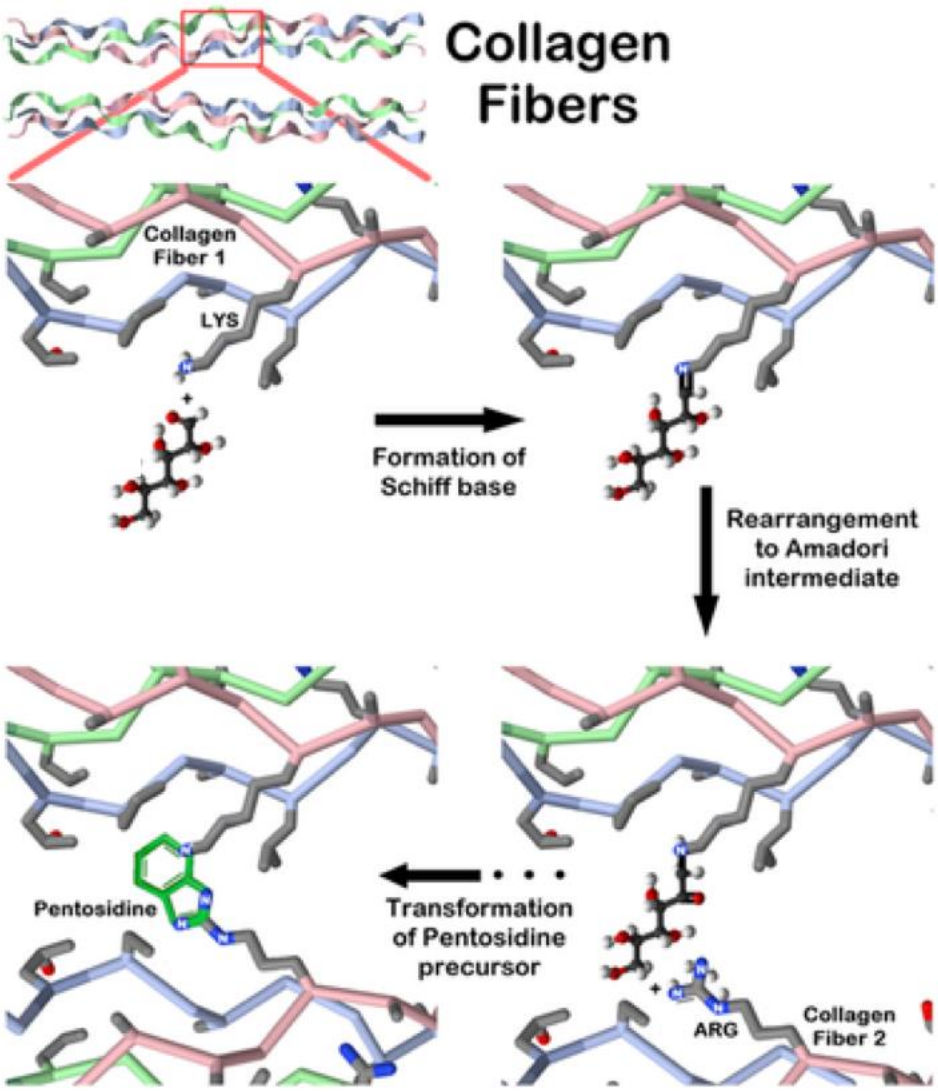


Figure 1-13 Schematic representation of the main steps of Maillard reaction.

In the initial step, a given sugar attaches to a free amino group present on the protein surface, and then, through a sequence of different reactions, an advanced glycation end product is formed. As the example, we show pentosidine as the final glycation product.(33)

Adapted from Sroga GE, Siddula A, Vashishth D (2015) Glycation of Human Cortical and Cancellous Bone Captures Differences in the Formation of Maillard Reaction Products between Glucose and Ribose. PLoS ONE 10(2): e0117240. doi:10.1371/journal.pone.0117240

The autonomic nervous system in diabetes

Animal studies have suggested that there is a hypothalamic control of bone mass regulated by leptin and mediated by the sympathetic nervous system (51). The activation of the sympathetic signalling related to stress have been associated with low BMD in human and

animal models (51). Furthermore, the reflex sympathetic dystrophy, a human disease characterized by localized increase in sympathetic tone and bone loss, is another evidence of the influence of autonomous nervous system (ANS) in bone mass (51). In addition, retrospective and prospective studies suggested a reduced fracture risk and increase in BMD in beta-blocker users, but data is still unclear (51). These findings suggest a role for autonomic innervation in bone homeostasis.

Diabetic autonomic neuropathy (DAN) is a common complication of diabetes. The prevalence varies widely, according to the reference population and the methods used to establish the diagnosis (52). A community-based population study in Oxford, which defined DAN as abnormal heart rate variability in one or two test results, described a prevalence of 16.7%. Autonomous nervous system may be affected in any organ, including the control of micro vascular blood flow (52). Therefore, innervation may influence bone health by a direct effect of sympathetic autonomous system or by an indirect effect in the regulation of bone blood flow. Both of them could be compromised by autonomic diabetic neuropathy.

Bone vascularization and diabetes

The development of MVD in diabetes is related to the inability of endothelial cells to regulate glucose transport. Once exposed to extracellular hyperglycaemia, they develop intracellular hyperglycaemia that has deleterious effects. Before structural changes are evident, abnormalities in blood flow and vascular permeability are detected at retina, glomerulus and peripheral nerve vasa nervorium. Hyperglycaemia leads to micro vascular hypertension and increase in the vascular permeability and then irreversible micro vessel occlusion. The progressive narrowing and occlusion of the lumens are followed by vascular and local cells loss (53). Bone tissue homeostasis is closely related to endothelium. Bone remodelling occurs throughout lifespan and both osteoclast and osteoblast develop from precursors that come through the endothelium. Micro vascular supply involvement may affect the highly regulated remodelling process, resulting in decrease in bone formation and micro damage repair (54). Microarchitecture was negatively affected in T2D with MVD but not in patients without MVD suggesting that abnormalities on bone vascularization might play a role.

Summary

In summary, it is known that overall there is an increase in the risk of fractures in diabetes. However, it is not known if this risk is affected by skeletal site, gender, age or BMI. Furthermore, it is not known if diabetes type, duration, complications and treatment will impact the risk.

In addition, the mechanisms associated with bone fragility in diabetes are not established. There is some evidence suggesting abnormalities in bone microarchitecture, but results are inconsistent. Some studies have suggested an influence of metabolic control and microvascular complications, but data are unclear. Therefore, it is important to investigate if there is an increase in the risk of fractures at the wrist and ankle, the sites assessed by HR-pQCT. In addition, it is important to investigate the effect of diabetes and microvascular complications on microarchitecture at these sites. This information could help to clarify the mechanisms associated with bone fragility in diabetes and if microarchitecture is an important feature.

Aims and objectives

The aims of this thesis were to assess fracture risk in diabetes and to investigate bone microarchitecture in peripheral sites in diabetes.

Chapter 3

The aim of this systematic review and meta-analysis was to assess the risk of hip and non-vertebral fractures in adults with diabetes compared to adults without diabetes in observational studies. Additional aims were to assess if gender, age, BMI and diabetes-related features such as DM type, duration, insulin use and the presence of complications affect this risk.

Chapter 4

The aim of this systematic review was to evaluate the risk of ankle and wrist fractures in diabetes

The aim of this study was to investigate if there is an impact of T1D on bone mineral density and microarchitecture at peripheral sites and if this impact was influenced by the presence of neuropathy.

In summary, this thesis summarised the risk of fractures in diabetes and investigated if bone peripheral microarchitecture was associated with bone fragility in diabetes.

Chapter 2 Methods

Section 1- Systematic review and meta-analysis methods

Section 2 - T1D and bone clinical study methods

Chapter 2 Methods

Section 1 Systematic review and meta-analysis methods

Introduction

This thesis reports the result of two systematic reviews on the risk of fractures in diabetes. One review investigated the risk of hip and non-vertebral fractures and the other investigated the risk of wrist and ankle fractures in diabetes. The specific methods used in each review will be described later in the respective chapters (chapters 3 and 4). This chapter will discuss the principles of systematic review methods and how they were applied.

What is a systematic review?

According to the Cochrane glossary a systematic review (or systematic overview) is

> **"**A review of a clearly formulated question that uses systematic and explicit methods to identify, select, and critically appraise relevant research, and to collect and analyse data from the studies that are included in the review. Statistical methods (meta-analysis) may or may not be used to analyse and summarise the results of the included studies." {Higgins, 2011, Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0;Higgins J, 2011, Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011].}.

Systematic reviews summarise the available evidence on a topic in a systematic way. The aim is to gather high quality evidence while minimising the risk of bias and random errors. The methodology should be clearly described to allow other people to reproduce the review. The review objectives and the eligibility criteria for studies inclusion should be pre-defined. Searches should be broad enough to try to identify all studies that would meet the eligible criteria. Once selected, studies need to be assessed for the validity of their findings and the risk of bias. The information gathered should be collated, analysed, summarised and described in a clear way. A qualitative analysis should always be reported (narrative synthesis) and on the availability of suitable data, a quantitative analysis (meta-analysis) should be performed (55).

Rationale

Systematic reviews and meta-analyses are considered the highest level of evidence in research, due to several features. Firstly, these studies are usually able to report data from large samples. By pooling together many studies it is possible to reach sample sizes that could not be reached in individual studies. In addition, gathering all the available evidence together allows the analysis of how much that topic has been explored and in how much depth. It is possible to acknowledge which information is available and determine if there is enough evidence to answer questions and make conclusions. If the information is not available, the review makes it clear what information is lacking. This is particularly helpful in guiding the next research steps. Furthermore, the method reduces the influence of single study problems such as flaws or errors. As many studies are polled together, the effects of flaws and errors is diluted. Finally, systematic reviews allow the possibility of new insights into the topic, that might come from gathering evidence from different sources and analysing them together (55).

How was it done?

In order to make the process transparent and reproducible, the systematic reviews were conducted following a structured method. Reviews were carefully planned, conducted and reported. Before the review, the rationale was assessed and the research question established. A protocol was developed and the reviews were conducted following high standards, according to the pre-established strategies defined in the protocol. Finally, the reviews were also reported in a systematic way.

Review question

Systematic reviews are conducted to answer research questions. The question should be clear and broad enough to justify conducting a review. There are some key principles that guide the development of the research question. These key principles are summarised by the acronym PICOS or PECOS, for reviews of observational studies. The acronym refers to P- participants; I- intervention, or E- exposure, in reviews of observational studies, C- comparator, O- outcomes and S- study design.

Initially, the participants need to be clearly defined. It is important to state which population will be investigated. Several populations have different characteristics that might influence

the results of an intervention or exposure. For example, in both reviews in this thesis, the participants were adults. The epidemiology of fractures and the risk factors are different in children and adults. When the population was defined as adults, the question was focussed on this specific population. During the search process, we found some studies that included data in regard to children in their overall analyses. These data could not be included, as they were considered out of the scope of these reviews.

The second letter of the acronym refers to the intervention, or exposure. Diabetes is an exposure that might be associated with fractures. For this reason, the PECOS acronym was applied. In the reviews described in this thesis, we investigated the risk of fractures in people with diabetes mellitus, defining diabetes mellitus as the exposure. We did not specify diabetes type, therefore we collected data from type 1 and type 2 diabetes, but we only collected data about the risk of fractures in this specific disease.

The third letter of the acronym refers to the comparator. The reference group should be clearly established. Several groups will have different baseline characteristics, and how much an intervention or exposure would modify one feature is directly related to which group is used as a reference. It is possible to assess multiple comparators; the main reference group will be addressed in the primary outcome and additional comparison groups, if applicable, will be addressed by secondary outcomes. However, the comparators should be established in advance in the protocol. In the reviews reported people without diabetes constituted the main reference group. The main aim of the reviews was to report the risk of fractures in adults with diabetes compared to adults without diabetes. The peripheral fractures review reported just this main comparator. Conversely, the hip and non-vertebral fractures review considered people without diabetes as the main comparator group, but a second comparator (the comparison between people with type 1 and type 2 diabetes) was also considered. However, as it was not possible to anticipate if enough data would be available, the protocol describe that this second comparison would be only done if possible.

The following letter is O, for outcome. The review question should address a specific outcome. The question needs to state clearly what the outcome of interest is to avoid any misunderstanding and to allow a clear definition of the research strategy. In this setting, the outcome was fractures, however, it was different in each of the reviews conducted. One review addressed the risk of wrist and ankle fractures, while the other review addressed the risk of hip and non-vertebral fractures. Although both of them addressed the risk of fractures,

the clear definition of which fractures would be included resulted in two independent processes; one specifically about the risk of peripheral fractures in diabetes and the other about the risk of hip and non-vertebral fractures in this disease.

Finally, the design of the studies to be included in the systematic review should be defined. Commonly, systematic reviews investigate the effect of interventions and randomized controlled trials are the preferable design. However, as diabetes is an exposure, it is not possible to investigate the effect of the disease on the risk of fractures through randomized controlled trials. The effect of exposure is usually described in observational studies. Both reviews focused on observational studies. Once the review question is clearly defined and the PECOS characteristics established the inclusion criteria for the review are defined (55).

Exclusion criteria should also be established to make sure only the suitable information will be included. In the reviews reported in this thesis, we excluded papers where the diagnosis of diabetes was made following the fractures or where the sequence between the diagnosis and the fracture was not clear. This was important to ascertain the association between the fracture and diabetes. In the hip and non-vertebral fracture review, only studies that included risk estimates adjusted for age and gender or single gender studies were included.

The protocol

The protocol is the review plan. It includes the background information, the research question to be assessed and the methodology of the review. The protocol makes the review process transparent and reproducible. It should be defined before the review and it will state the rules that will be followed during the review process. It is important that these rules are developed *a priori* to avoid the risk of changing the strategy motivated by the data collected. The protocol also allows standardisation of the process, especially when reviews are conducted by a team.

The protocol establishes the review methods such as the literature searches, the study selection process, including the eligibility criteria, the quality assessment, the data extraction strategy and the data synthesis. While developing the protocol the team should try to anticipate potential challenges that could be faced during the review process and how to deal with them (55).

Protocols were developed for both reviews reported in this thesis, but the process was not the same for each review. For the peripheral fractures systematic review, I developed the

protocol. For the hip and non-vertebral fractures review, the protocol was developed by ScHARR and discussed with the clinical team (myself, Richard Eastell). The process allowed the appraisal and contribution from both teams.

Protocols should be registered on the PROSPERO website. Registration aims to make the process transparent and reproducible. It also helps avoid the possibility of reviews addressing the same question being conducted simultaneously. Registered protocols can be amended, allowing some justified modifications, without compromising the review transparency. The peripheral fractures review protocol was not registered. The hip and non-vertebral fractures review protocol was registered in PROSPERO (protocol record number CRD42018090378) and subsequently amended, focusing the research in studies that report risk estimates adjusted for age and gender.

The review report

The review report describes the review process and results. Initially, the report includes the background information, introducing the topic and explaining the rationale for the review. Following this introduction, the report describes in detail the methodology used to keep transparency and to enable reproducibility. The report also describes the results of the review. The data collected is tabulated, described and analysed. Finally, the findings are discussed, including how the review contribute to the current literature, what the implications of the findings are, and what the proposed further steps to be investigated are (56).

The background information

Background information is important to contextualise the review. This section introduces the condition or the disease explored and establishes the rational for the review.

Although both reviews explored the risk of fractures in diabetes, background information was different for each review. Hip and non-vertebral fractures, reported in one of the reviews, are fractures associated with osteoporosis. These fractures are end points commonly reported in clinical trials. In regard to the other review, wrist fractures are also associated with osteoporosis, but not ankle fractures. Ankle fractures are not considered typical osteoporotic fractures. In addition, the epidemiology of all these fractures is diverse, something which was addressed in each specific review's background information section.

Review methodology and results

Search strategy

The search strategy includes the definition of the study sources, the terms to be used in the search and the limits to be applied. Several databases can be used for electronic search, such as MEDLINE, Embase and The Cochrane databases. Conference abstracts and reference lists of key papers can also be a source of studies. The key terms should be selected including free-text and thesaurus terms (where available) and combined using Boolean operators. Any limits such as date, human or animal studies or language can be applied (55).

Both reviews combined terms for fractures and diabetes mellitus and related synonyms, but they followed different search strategies. In the peripheral fractures review, the search was focused on wrist and ankle fractures, but the remaining search limits were broad. No restriction in regard to date and language were applied. In addition, conference abstracts were also searched adding grey literature as a source of data. Conversely, the hip and non-vertebral fracture review was planned as a review update. Databases were searched from inception to find systematic reviews on fracture risk in diabetes. Several reviews were found. They were assessed and one review was selected as the baseline review to be updated. The search for original papers then started from the date when the searches of the baseline review were conducted. Searches were restricted to English. For both reviews we searched references list from key papers in the field.

Study selection

The study selection follows a systematic flow. The retrieved records are collated. The first step in the process is the removal of duplicates. The following steps will assess the records against the inclusion and exclusion criteria described in the protocol. Firstly, records are assessed on the basis of title and abstract, which results in the exclusion of the majority of the clearly irrelevant papers. Subsequently, the remaining records undergo full text assessment.

In both reviews, the initial title and abstract selection was done by a single reviewer. In the hip and non-vertebral fractures review, a second reviewer sift a 10% random sample of the retrieved records and the kappa statistics was calculated for both the systematic reviews

sifting and the original papers sifting. The full text sifting was done by one reviewer in the peripheral fractures review and by two reviewers in the hip and non-vertebral fractures review. The selected papers were included in the review and underwent quality assessment and data extraction.

Quality assessment

Quality assessment is important to evaluate the internal and the external validity of the studies. The internal validity will address the risk of bias and the external validity will address the generalisability of the results. The quality of the results reported in any review depends on the quality of the data included. Multiple aspects of the study might be affected by bias such as the selection of the participants, the performance of the studies, the detection of the outcome of interest and also participant's attrition. In observational studies, the selection bias can be minimised by the control for confounders. Bias in the performance of the studies could be minimised by adequate measurement of the exposure. Bias in the detection of the outcome should be minimised by appropriate detection and blindness of the outcome assessment. Finally, attrition bias is minimised by completeness of the follow-up.

The Newcastle-Ottawa scale is a tool to assess the risk of bias in observational studies. The studies are awarded stars if they comply to each feature assessed to a maximum of nine. Studies that score seven or more are considered high quality. There are specific scales for cohort and case-control studies. The scale includes three domains; selection of participants and comparability (common to both cohort and case-control scales) and assessment of the outcome (for cohort studies) or assessment of the exposure (for case-control studies). In the comparability domain, the scale should be adapted to each review. The adaptation aims to assess if the study controls for the most important factor and for an additional factor.

The Newcastle-Ottawa tool was used to assess the quality of the studies included in both reviews. In these reviews, age and gender were selected as the most important factors to be controlled for. Consequently, studies were awarded one star if they controlled for age and one if they controlled for gender.

Data extraction

Data should be extracted using standardised forms that should be piloted. Data extraction should be checked. In the peripheral fractures risk review, data was extracted and checked

by the same reviewer, while in the hip and non-vertebral fractures review, data was extracted by one reviewer and checked by a second.

Data Synthesis and reporting

Systematic reviews should include a qualitative synthesis and might also include a quantitative synthesis, the meta-analysis. Both the reviews described in this thesis were reported following the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guideline. The PRISMA is an evidence-based guideline that lists the minimum items that should be reported in systematic reviews and meta-analyses. The tool was designed to be used in reviews that summarise the findings of randomized trials, but it can also be applied to reviews of observational studies. The PRISMA reporting strategy suggest the use of a flowchart to describe the research process. The flow chart summarises the study selection process. The figure describes the number of studies screened and excluded in each phase of the process, the number of studies assessed for eligibility and also the number of studies included in the review. The PRISMA flowchart was used in both the reviews reported (Figure 3-1 Figure 4-1) (93).

The PRISMA guideline also includes a checklist with the main items that should be included in the title, abstract, introduction, methods, results and discussion. Both the reviews reported were checked against PRISMA checklist.

Qualitative synthesis

In the qualitative synthesis, data is reported including the tabulation of the study characteristics and a narrative synthesis that summarise the data across studies. The quality assessment should also be tabulated and narratively described.

In both reviews, we described the data using tables and a narrative report. The key features of each study were described in tables (Table 3.2, Table 3.5, Table 4.1, Table 4.2). In addition, we also include a narrative report, summarising the main findings. Results of the quality assessment were described following the same pattern.

Quantitative synthesis

In case there is available data, quantitative synthesis should be performed and studies should be summarised in a meta-analysis. Meta-analyses are weighted averages of the effects

estimates that pool together the results of the individual studies in a summary number. Summaries are more robust evidence than individual studies.

Two models can be used to combine studies. The fixed-effect model assumes that the studies report the same effect size and any potential differences will be due to the variations in each sample. Conversely, the random-effects model assume that the true effect size might vary between studies, but this variation follows a normal distribution. The random-effects model considers the variation of the effect size measured due to variations in each sample and also the variation in each study (55).

The data summarised reported the risk of fractures in multiple settings, such as DM types, age ranges, diabetes duration and several sources of patients. In addition, the studies were also methodologically different. There were cohort studies (some prospective and others retrospective) and case controls studies. In some studies data came from registries and in others the participants were recruited. It was expected that the effect found in the studies would differ and, for this reason, we have used the random-effects model.

Clinical features could also influence the risk of fractures. Subgroup analyses should be planned to explore the effect of clinical features that could potentially influence the outcome, in this case, the fracture risk. In the diabetes scenario, age, gender, BMI, diabetes type, insulin use and the presence of microvascular complications are clinical features that could potentially influence the risk of fractures. In addition, clinical features might affect the risk of fractures in different ways. For example, obesity is reported to decrease the risk of hip and other osteoporotic fractures but it is reported to increase the risk of ankle fractures. This discrepancy highlighted the importance of a subgroup analysis for BMI. Subgroup analyses addressing the features listed above were anticipated in the hip and non-vertebral fractures review. It is worth mentioning that we could anticipate that these features could potentially affect the risk of hip and non-vertebral fracture but we could not anticipate if we would find enough data to perform the analyses. The peripheral fractures review did not anticipate any subgroup analysis.

Sensitivity analysis can also be programmed to explore the influence of other features in the analyses. The influence of a specific study, or of a group of studies, with a given characteristic can be explored. These analyses investigate the impact on the risk estimate and also on the heterogeneity. In both reviews, sensitivity analysis excluding one study at a time was

conducted to investigate the effect of each individual study. We also conducted sensitivity analysis excluding studies not considered high quality in the quality assessment.

The variation across the studies is reported by the heterogeneity. The random-effects model allows for this heterogeneity, as the variation in the effects size between studies is considered. As the studies included in these meta-analyses are quite diverse, high heterogeneity could be found. Subgroup and sensitivity analyses and meta-regression were used to explore the heterogeneity. Subgroup analysis clusters the study in specific subgroups (for example diabetes type) and investigates if there are significant differences between these subgroups. Sensitivity analysis explores the impact of studies with a given characteristic in the results, by excluding these studies. Finally, meta-regression explores how much the variation in the results is due to one or more specific features.

In the peripheral fractures meta-analysis, we did not anticipate subgroup or sensitivity analyses. In the hip and non-vertebral fractures analysis, subgroup and sensitivity analysis were planned and conducted. As high heterogeneity was found, meta-regression was also conducted.

Publication bias

Systematic reviews and meta-analysis use mainly published papers as a source of data. Larger studies and studies with larger effects are more likely to be published. Conversely, small studies with no or small effects are less likely to be published. Some tools have been developed to investigate if there is a relationship between sample size and effect size, also called the small studies effect. This relationship might be a sign of missing studies. Funnel plots are widely used to investigate this relationship. A funnel plot is a scatter plot of the effect estimate from individual studies against some measure of the study size. The standard error is often used and plotted in a vertical axis with a reverse scale. If the effect is the same in each study (fixed effects model assumption), a triangle centred on a fixed effect summary estimate and extending 1.96 SD each side will include about 95% of the studies. If there is no bias or heterogeneity, the plot will resemble a triangle, as the scatter will be due to random sample variation. This would be a symmetrical funnel plot. A number of reasons can cause asymmetry such as reporting bias (due to publication bias, selective outcome or selective analysis reporting), poor methodological quality leading to spuriously inflated effects in smaller studies, true heterogeneity, artefacts or chance. Tests for small studies effect

investigate whether the association between the study effect and the study size is greater than it would be expected to occur by chance. The Egger's and Begg's tests are examples. If the small studies effect is detected and publication bias is suspected, the Trim and Fill correction can be applied. The method removes small studies causing asymmetry, estimate the number of missing studies and add them and the estimates of their effects. Consequently, the method provides a RR for a symmetrical funnel plot, as if there was no publication bias (93).

In both reviews, visual analysis of funnel plots was used to assess publication bias. In the non-vertebral fractures' analysis, publication bias was suspected. The funnel plot was asymmetric with an empty spot at one side and there was no important heterogeneity (another cause for asymmetry in funnel plots). Hence, the Trim and Fill method was applied.

The "trim and fill" method is a tool used to identify funnel plot asymmetry arising from publication bias and to correct this asymmetry. The tool assumes that the asymmetry is caused by publication bias, but takes no assumption in regards to the mechanisms of this publication bias. The method is used to remove ("trim) the smaller studies leading to funnel plot asymmetry. The trimmed funnel plot is used to estimate the true "centre" of the plot and to estimate the number of missing studies around the centre. These missing studies and their adjusted intervention effect are added to the calculations ("fill"). The tool provides an estimate of the number of missing studies and the estimated intervention effect adjusted for the publication bias. However, it considers that there should be a symmetric funnel plot (what is not always the case). In addition, it is not possible to assess if the adjusted intervention effect matches what would have been observed in the absence of publication bias, especially because the mechanism of the publication bias is unknown. Finally, the trim and fill method does not consider other reasons for asymmetry besides publication bias. Therefore, results "corrected" by this method should be interpreted with caution. (57)

Most of the recommendations about funnel plots are designed to meta-analyses of randomised trials and whether they apply to meta-analyses of epidemiological studies in unclear (93).

Discussion

In the discussion, the key findings are summarised and related to the current literature. The implications of these findings with regards to practice and polices and the strengths and weakness of the review are discussed. Finally, the areas to be explored by further research should be suggested. Both reviews included a discussion section.

Section 2 T1D and bone clinical study methods

Study design

This was a single-centre, observational, cross-sectional, case-controlled study to evaluate the effects of type 1 diabetes mellitus and diabetic neuropathy on bone health in patients with T1D. T1D patients with and without diabetic neuropathy will be compared to each other and to healthy controls.

Participants

Participants with T1D were recruited from Sheffield Teaching Hospital outpatient clinics and Diabetes Database between October 2017 and October 2018. Participants were patient with diabetes, men and women older than 18 years, with more than 5 years of T1D diagnosis, without CKD (eGFR > 60 ml/min.m^2) with and without diabetic neuropathy and healthy volunteers. Healthy volunteers were recruited from the Bone Research Unit database and from emails sent to the Trust employees. This resulted in three groups: T1D with neuropathy (T1DN+), T1D without neuropathy (T1DN-) and control. Since gender and age affect bone structure, we tried to match participants as close as possible by these two features. As skeletal size affects BMC in DXA and the site to be scanned by the HR-pQCT, we tried to match participants as close as possible by height. The initial plan was to match participants in a 5-year interval and a 5 cm interval to minimise the effect of potential confounders.

We attempted to individual match, but recruitment was a challenge and the desirable targets could not be achieved. Consequently, we opted to the conservative approach and the analysis was comparison by groups.

This study was approved by Liverpool Research Ethics Committee (IRAS 222726, 17/NW/0291). All participants provided written informed consent, in accordance with Good Clinical Practice guidelines.

Sample size

Power calculations were used to calculate the sample size. The standard deviation from previous studies that have reported the cortical porosity in T1D was used (3.03%) (25) and the clinically significant difference was estimated in 3.0%. This was the difference in cortical porosity previously reported between patients with diabetes with and without fractures (29).

This resulted in a sample size of 20 in each group. This sample size has 80% power to detect a difference of 3.09% in cortical porosity at $p<0.05$.

Inclusion and Exclusion criteria

Inclusion criteria

- Male and female participants aged 18 or older;
- Sufficiently mobile to undergo scanning;
- Able to remain motionless for the duration of the scans;
- Able and willing to participate in the study and provide written informed consent;
- Participants with diabetes: Patients with type 1 diabetes with more than 5 years of T1D diabetes diagnosis, without CKD (eGFR > 60 ml/min.m^2). They will be evaluated and classified according to the presence of diabetic neuropathy.
- Healthy controls: Haemoglobin A1c levels (HbA1c) less than 5.7% (39 mmol/mol), according to American Diabetes Association standards.

Exclusion criteria

- Previous orthopedic surgery or fractures which preclude imaging at all sites;
- History of any long-term immobilization (duration greater than three months);
- High or low trauma fracture less than one year prior to recruitment;
- History of bilateral fractures at tibia and/or radius;
- Current pregnancy or trying to conceive;
- Delivery of last child less than one year prior to recruitment;
- Breast feeding less than one year prior to recruitment;
- Women in the perimenopause period, including 5 years after menopause;
- History of or current conditions known to affect musculoskeletal health, diabetes and/or neuropathy evaluation or bone metabolism including:
    - Diagnosed skeletal disease
    - Osteoarthritis at study measurement sites
    - Chronic renal disease
    - Malabsorption syndromes

        - Hypocalcemia or hypercalcemia
        - Diagnosed restrictive eating disorder
    - Conditions which prevent the analysis of the DXA scans or the interpretation of their results;
    - Conditions which prevent the analysis of the HR-pQCT scans or the interpretation of their results;
    - Use of medications or treatment known to affect musculoskeletal health, diabetes and neuropathy evaluation or bone metabolism including depot medroxyprogesterone or the combined oral contraceptive pill;
    - Alcohol intake of greater than 21 units per week;
    - Markedly abnormal clinical laboratory parameters that are assessed as clinically significant by the Principal Investigator.
    - For healthy controls, abnormal levels of fasting glucose (fasting glucose levels > 5.6 mmol/L) or HbA1c > 5.7% (39 mmol/mol).

Inclusion and exclusion criteria were designed to guarantee a number of features. Firstly, participants needed to be willing to take part on the study. Furthermore, the participants with T1D should have the disease for sufficient time to be affected by potential adverse effects on bone and healthy volunteers should not have diabetes. In addition, participants should not be affected by conditions that affect bone structure or bone turnover. Finally, to ensure the quality of the scans, participants with conditions that prevent the adequate acquisition and analysis of the images were excluded, in order to make the images reliable and interpretable.

Study procedures

All participants attended two visits. For the participants with T1D, the first visit was at the Diabetes Research Clinic at the Royal Hallamshire Hospital (RHH), where the neuropathy assessment was conducted. The healthy volunteers could schedule the first visit to the RHH or the Clinical Research Facility (CRF) at the Northern General Hospital (NGH), according to their convenience. All the participants attended the second visit at the CRF, NGH.

Table 1.1**Error! Reference source not found.** list the procedures undertaken in each visit

Table 2.1 Study visits and procedures

Visit	Procedure
Visit 1	Informed consent, Blood sample, Height, weight and BMI, Neurophysiology evaluation*
Visit 2	Height, weight and BMI, Pregnancy test (premenopausal women only), DXA (lumbar spine, proximal femur, HR-pQCT

* T1D participants

Visit 1

Blood samples

Blood samples were collected for screening tests (PTH, calcium, creatinine, HbA1c).

Intact PTH (second generation) was measured using an immunoassay method by the Roche Cobas 8000 e602 (Roche Diagnostics GmbH, Mannheim, Germany). The interassay coefficient of variation (CV) measured in the laboratory is 2.2 – 3.2% at 34 ng/L, 1.6 – 1.7% at 94 ng/L and 1.4 – 1.8% at 839 ng/L, while the reported reference interval is 15-65 ng/L (1.6 - 6.9 pmol/L).

Serum calcium was measured using a Roche/Hitachi Cobas 8000 e702 automated clinical chemistry analyser (Roche Diagnostics GmbH, Mannheim, Germany). This method uses 5-nitro-5'-methyl-BAPTA (NM-BAPTA) reagent. The interassay coefficient of variation as measured in the laboratory is 1.1 – 1.5% at 1.52 mmol/L and 0.6 – 1.1% at 3.07 mmol/L. Albumin measurement was performed using a Roche/Hitachi Cobas 8000 e702 analyser (Roche Diagnostics GmbH, Mannheim, Germany). The interassay coefficient of variation as measured by the laboratory is 1.5 – 2.4% at 33.9 g/L and 1.0 – 1.7% at 59.7 g/L.

The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to estimate eGFR.

$$eGFR\left(\frac{mL}{min \times 1.73\ m^2}\right) = 141 \times \min\left(\frac{Scr}{k}, 1\right)^{\alpha} \times \max\left(\frac{Scr}{k}, 1\right)^{-1.209} \times 0.993^{age} \times 1.018\ [if\ female] \times 1.159\ [if\ black]$$

Scr is serum creatinine in mg/dL, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κ or 1, max indicates the maximum of Scr/κ or 1

The aim was to exclude calcium abnormalities and chronic kidney disease. For the healthy volunteers, diabetes was also excluded.

Anthropometry

Weight and height were measured in shoeless participants wearing light clothes to confirm eligibility (matching).

Neurophysiology evaluation

According to the Toronto Consensus meeting, DSPN is defined as length-dependent sensorimotor polyneuropathy, that is chronic, symmetrical and associated with longstanding hyperglycaemia and cardiovascular risk factors (6). Abnormalities in nerve conduction studies associated to signs and or symptoms of neuropathy are required to confirm the diagnosis (6). In order to categorise the T1D participants into the neuropathy and non-neuropathy groups we used standard questionnaires to investigate symptoms and a number of tests were performed. Small fibres were assessed thorough Sudoscan and the Toronto Clinical Neuropathy Score. Large fibres were assessed by nerve conduction studies and the Toronto Clinical Neuropathy Score.

Sudoscan

The sweat glands are innervated by sudomotor thin postganglionic unmyelinated cholinergic sympathetic C-fibers, also called small fibers. Sudomotor disfunction is one of the earliest abnormalities detected on distal small fiber neuropathy. Skin biopsy studies have shown a decrease in the number of these fibers in people with diabetes (58). SUDOSCAN is a non-invasive quantitative assessment of sudomotor function. The device uses low voltage current to stimulate the sweat glands. The participants placed palms of their hands and the soles of their feet on the stainless-steel electrodes (Figure 2-1). The sweat produced contains chloride ions which react electrochemically with stainless steel electrodes. The ratio between the current that is measured and the voltage applied is used to calculate the electrochemical skin conductance (ESC), and a measure of sudomotor function expressed in microsiemens (μS). It

generates to measurements, hands electrochemical skin conductance (HESC) for the hands and feet electrochemical skin conductance (FESC) for the feet. The report suggests no risk of neuropathy, moderate risk of neuropathy or elevate risk of neuropathy (58). Several studies have shown good reproducibility with the method (58-60). Reproducibility was not assessed in this study.

Figure 2-1 Sudoscan assessment (58)

We used the Toronto Clinical Neuropathy Score (TCNS) to assess DSPN. The TCNS is a simple comprehensive method to evaluate DSPM. The assessment includes signs and symptoms from small and large fibres. The TCNS has been validated against nerve conduction velocities and amplitudes (61) and morphological criteria of sural nerve fibre density (62). The tool assesses symptoms, reflexes and sensory test in both limbs, as described on Table 2.2. The presence of abnormalities is graded by scores to a maximum of 19. The symptoms scores are assessed by the examiner in each limb as present (1) or absent (0). The sensory tests are graded as abnormal (1) or normal (0) and the reflexes as absent (2), reduced (1) or normal (0). Patients are categorised according to the scoring in no neuropathy (0-5), mild neuropathy (6-8), moderate neuropathy (9-12) and severe neuropathy (>12). Previous studies have

reported an intra-observer variability of 7.3% and interobserver variability of 6.3% (62). Reproducibility was not assessed in this study.

Table 2.2 The Toronto Clinical Neuropathy Score (TCNS)

Symptom score	Reflexes	Sensory test scores
Foot Pain	knee	Pinprick
Numbness	ankle	Temperature
Tingling		Light touch
Weakness		Vibration
Ataxia		Position
Upper limb symptoms		

Nerve conduction studies

We used DPN check (Neurometrix, Waltham, MA, USA) to perform the sural nerve conduction assessment. The DPN check is a point-of-care device that assess nerve amplitude potential (sural nerve action potential - μV) and conduction velocity (m/s) using principles similar to the standard nerve conduction studies (63). Standard nerve conduction studies stimulate the nerve antidromically and requires the careful positioning of the probes over the sural nerve area to repetitively stimulate the nerve until a valid response is detected. While using the DPN check, stimulating probes are placed at the lateral region of the ankle and a biosensor, located 9.22 cm from the probes, covers the area above the ankle to record the responses (Figure 2-2 Figure 2-3) (64). The device stimulates the sural nerve orthodromically and the responses are collected in a wider area than in the standard nerve conduction studies. However, the device has been validated and demonstrated excellent reliability and acceptable accuracy in DSPN. In addition, intra-observer intraclass correlation coefficients were 0.97 for nerve amplitude potential and 0.94 for never conduction velocity and the interobserver was 0.83 and 0.79 respectively. (64). Participants were accessed on the two limbs. Reproducibility was not assessed in this study.

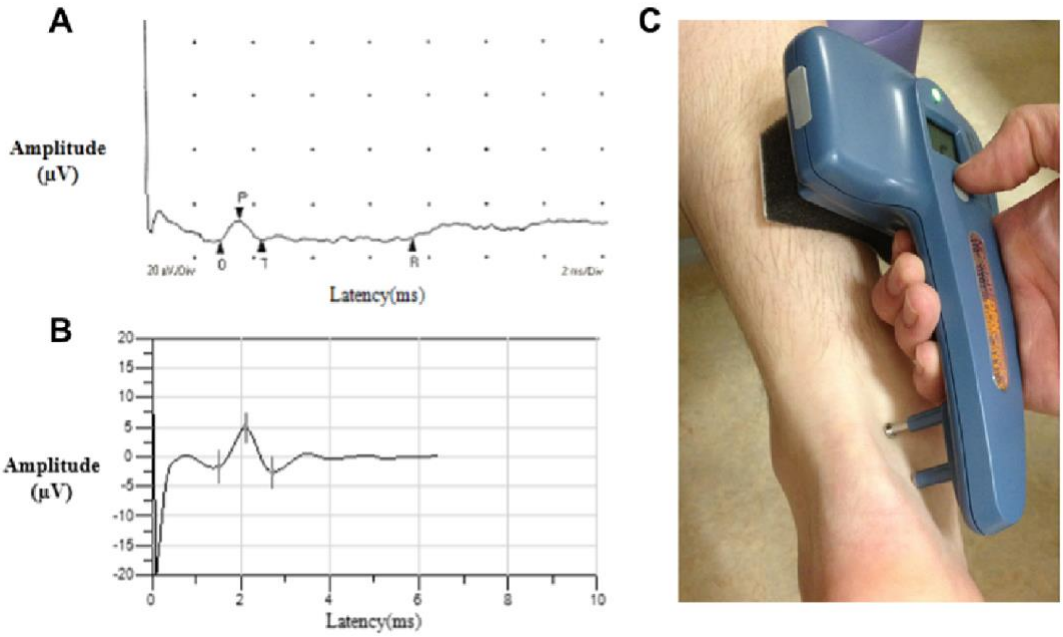


Figure 2-2 DPN check device in use

Sample nerve conduction recordings from standard NCS (A) and the point-of-care device (B) from a 60-year-old female with type 2 diabetes and an image of the point-of-care procedure (C). Panel A: Sample standard NCS recording. Sural nerve amplitude potential was 6.8 mV and conduction velocity was 48.3 m/s. Panel B: Sample recording from the point-of-care device. Sural nerve amplitude potential was 8 mV and conduction velocity was 56 m/s. Panel C: The device was placed on the lateral aspect of the leg and the sural nerve was stimulated and recorded by the electrical probes and biosensor, respectively (63).

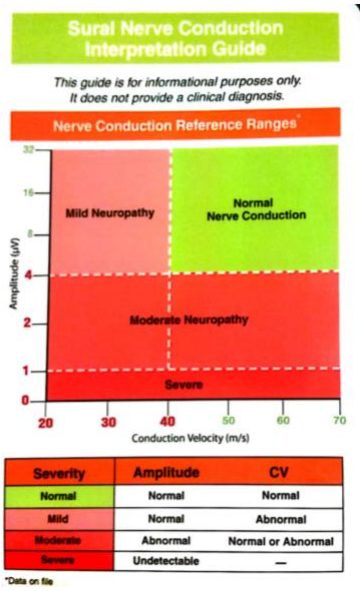


Figure 2-3 DPN check interpretation guide

T1D participants' group allocation

We used DPSN as the main feature to categorise participants in the neuropathy or non-neuropathy group. Participants with a TCNS indicating no neuropathy (score ≤ 5) and a normal nerve conduction study by DPN check were included in the no neuropathy group (T1DN-).

Participants with abnormal TCNS (score >6) and abnormal nerve conduction studies were included in the T1DN+.

Visit 2

Fasting blood samples

Sample collection

After overnight fast, blood samples were collected from each participant. Samples were allowed to clot at room temperature for 30 minutes before being centrifuged at 3000rpm for 10 minutes. Serum samples were aliquoted and stored at -80ºC until analysis.

Principles of chemiluminescence immunoassay

For the measurements of CTX and PINP, the IDS-iSYS was used. The IDS-iSYS is a chemiluminescence immunoassay (CLIA). It uses two antibodies; an anti-analyte antibody labelled with biotin and an acridinium labelled antibody. Once the serum sample is loaded onto the autoanalyzer, the two antibodies are added, followed by magnetic micro-particles coated with streptavidin that bind to the biotin in the complex. The mixture is incubated to allow magnetic particles to bound to a magnet. Then, a wash step removes the unbound substances. The acridinium conjugate is stimulated to emit light. The intensity of the light is proportional to the concentration of the analyte The CTX reflects an 8 amino acid sequence from the C-terminal region of type I collagen; the PINP reflects only the intact (not the total as it doesn't include the monomer) triple helix of the N-terminal propeptide region.

Dual energy X-ray absorptiometry

Scan acquisition and evaluation

Dual energy X-ray absorptiometry (DXA) was used to assess aBMD of the lumbar spine (L1-4) and hip (total hip and femoral neck) using a Discovery A densitometer (Hologic Inc.: Bedford, MA, USA) and Hologic software (version 12.6). DXA provides a two-dimensional projection of the anatomical site of interest from which area and BMD are measured. The aBMD is used to calculate scores that compare the individual measure with predefined populations and helps

in the assessment of the results. Previous studies have shown that BMD by DXA can predict fracture risk and is used as surrogate of bone strength (65, 66). The scores calculated from DXA can be used to define osteoporosis.

Principles of DXA

DXA is based on the principle that tissues would attenuate X-ray differently, according to their density. The method uses a low radiation dose. By alternating the voltage of the X-Ray tube, an X-ray source emits two distinct levels of energy: one high (140kVp) and one low (100kVp). The beams pass through a collimator that produces a fan beam. Tissues attenuate the X-ray beams differently according to their density: the denser a tissue, the more it will attenuate the X-Ray beam. Hence, some photons are absorbed and some scattered. A detector is placed opposite to the X-ray source and detects the energy difference of the two original beams. The human body is composed by several tissues which will attenuate the beams differently. Based on these differences, the device is able to detect bone, differentiate it from soft tissue and to determine bone area and BMD. The result is a two-dimension measurement and BMD is expressed in g/cm^2. However, this is not the most used value to assess BMD in the clinical setting. Usually, BMD is reported using scores.

Since BMD follows a normal distribution, means and standard deviations (SD) are calculated using population databases. The score places the individual value measured in the population data distribution and express this value in SD from the mean population in a given database. To generate the Z-score, a database from a population at the same age is used for comparison. Consequently, the Z-score expresses in SD how far the result is from the mean for the same age population. Conversely, the T-score refers to a population on the peak of bone mass (20-39 years), expressing how the results found differs from the mean of the peak bone mass population. The values measured by DXA are able to predict fractures and they are used to assess bone strength (65).

DXA procedure

The manufacture's standard procedures for each site were used to obtain DXA images, as previously described by our Research group (67). A Hologic Discovery A densitometer (Hologic Inc, Bedford MA, USA) was used to obtain scans in a posterior-anterior (PA) projection at lumbar spine and hip.

Hip

With the participant laid on the scan table in a supine central position within the scan limits, a head positioner was used to ensure the participant was comfort and well positioned. The foot on the scanned side was attached to a hip positioner to keep the leg abducted and the hip internally rotated by approximately 25°. Arms were kept away from the scan field, and placed on the chest (Figure 2-4). An express scan was used to assess positioning: at least 3 cm of straight femoral shaft bellow the lesser trochanter was included in the scan field. Positioning was corrected when necessary.

Figure 2-4 Participant's positioning for DXA proximal hip scan

For analysis, the global region of interest was positioned according to standard procedures; i) the lateral border was positioned 5 scan lines from the edge of the greater trochanter; ii) the medial border was positioned 5 scan lines form the edge of the femoral head; iii) the bottom border was positioned 10 scan lines bellow the lesser trochanter and iv) the upper border was positioned 5 scan lines from the edge of the femoral head. After correct positioning, the bone map was identified and the central axis of femur was used to place the midline and the neck box was positioned close to the great trochanter. The neck box also included equal amounts of soft tissue on each side of the femoral neck (Figure 2-5).

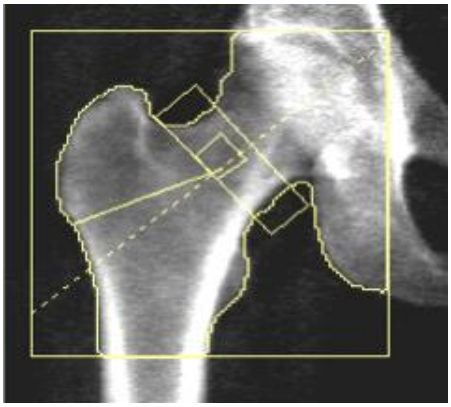


Figure 2-5 DXA proximal femur analysis – region of interest placement

Lumbar spine

For the lumbar spine scan, the hip positioner was removed and a spine positioning block used to elevate the legs (Figure 2-6). Scan image needs to be straight and central and the image should include from mid L-5 to mid T-12, as the global region of interest should include L1 to L4. An express scan was used to assess positioning before the scanning.

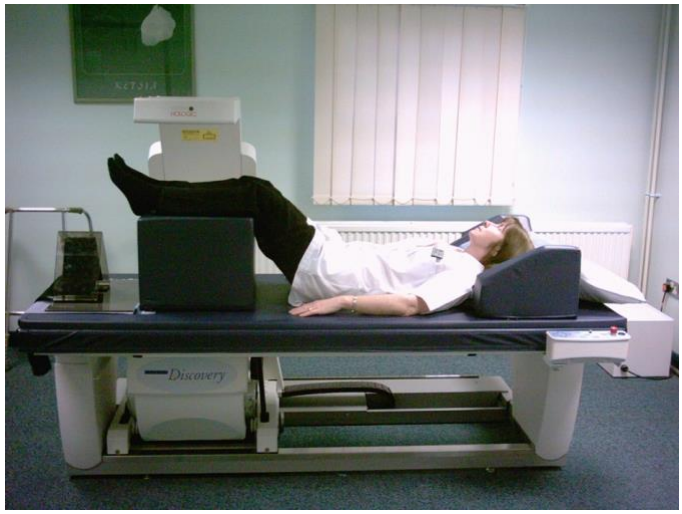


Figure 2-6 Participant's positioning for DXA lumbar spine scan

For interpretation, the top border was positioned within the T12-L1 intervertebral space and the bottom border within L4-L5 intervertebral space. If needed the borders could be angled to accommodate the shape of the vertebrae. Lines placed in each intervertebral space would identify each vertebra. Bone map was identified and corrected if necessary (Figure 2-7).

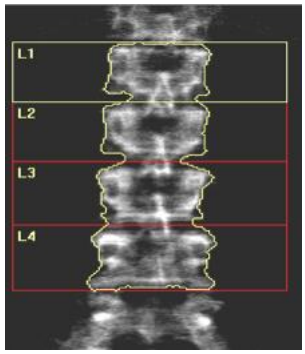


Figure 2-7 DXA lumbar spine analysis – region of interest placement

DXA quality control

All scans were performed by a highly trained operator. In accordance with manufacturer recommendations, quality control assessments were performed daily to ensure stability and precision. An anthropometric spine phantom containing four single density semi-hydroxyapatite 'vertebrae' was used for this purpose. In addition, the measurements were plotted in graphs against pre-specified acceptable limits set by the manufacturer. All of them (bone area, aBMD and BMC) were within the limits (Figure 2-8 Figure 2-9 Figure 2-10). The coefficient of variation (CV) were below 0.4%.

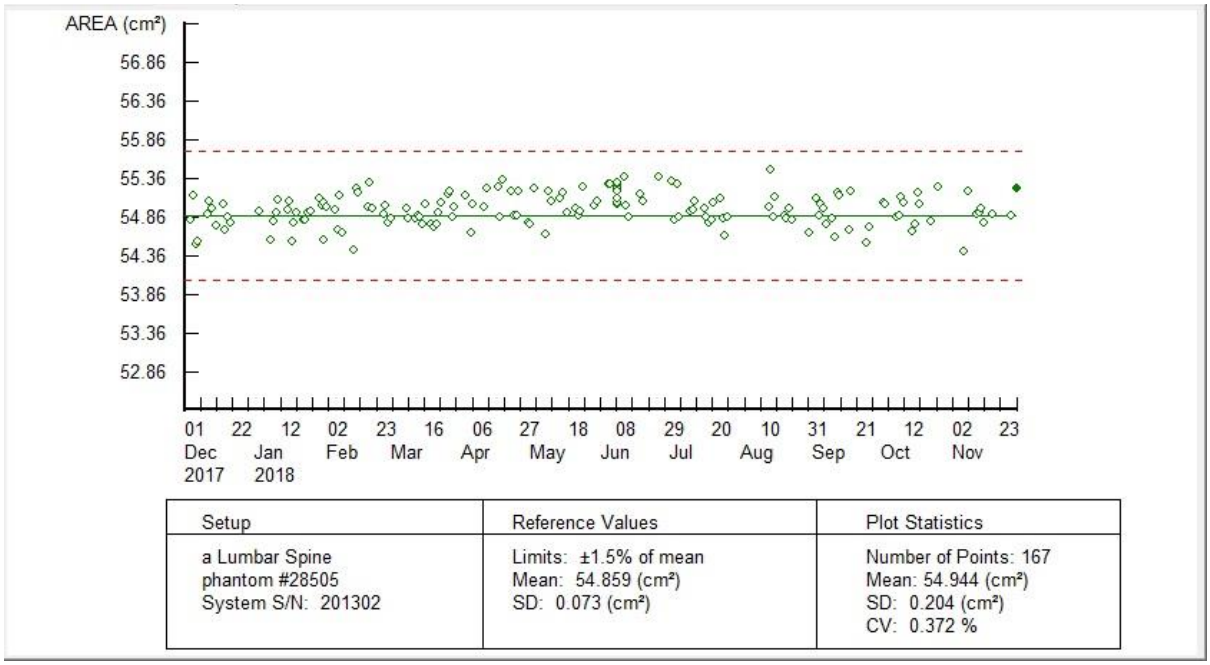


Figure 2-8 DXA quality control plot for bone area throughout the T1D and bone study

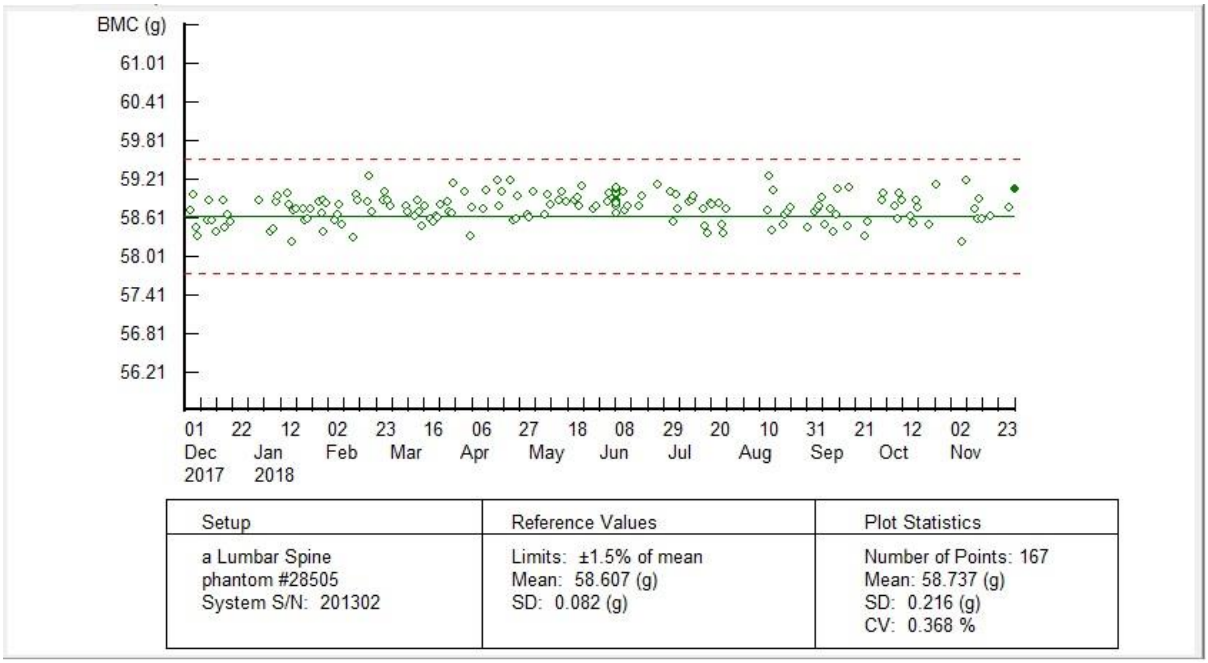


Figure 2-9 DXA quality control plot for BMC throughout the T1D and bone study

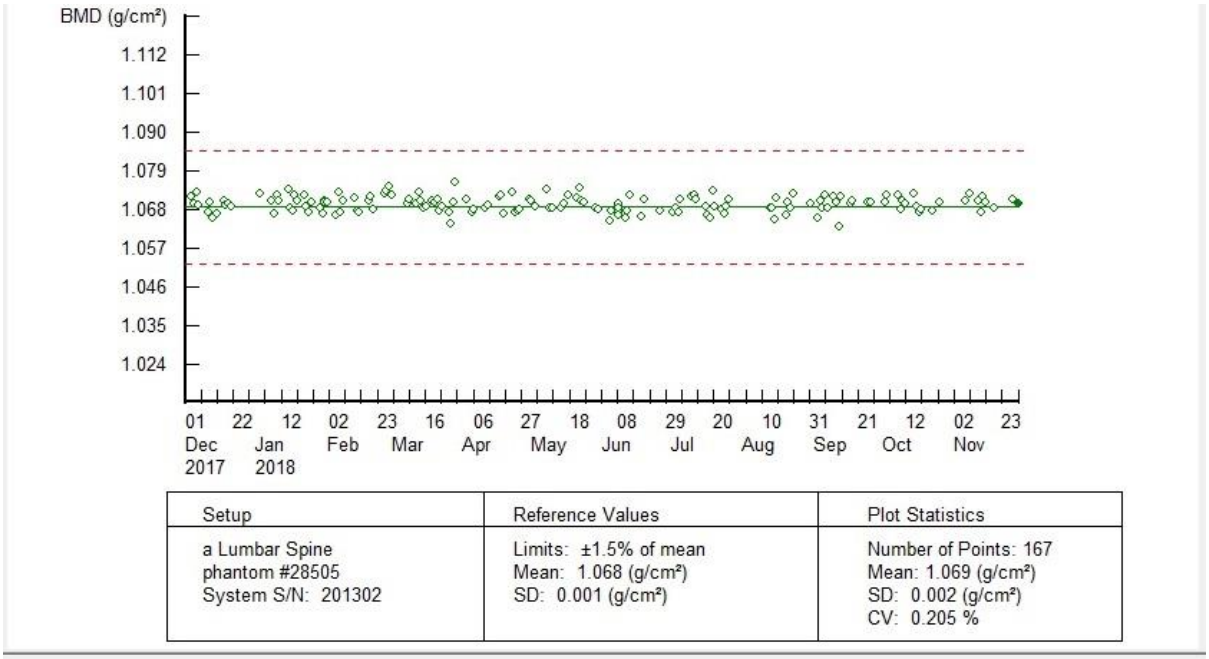


Figure 2-10 DXA quality control plot for BMD throughout the T1D and bone study

DXA precision error

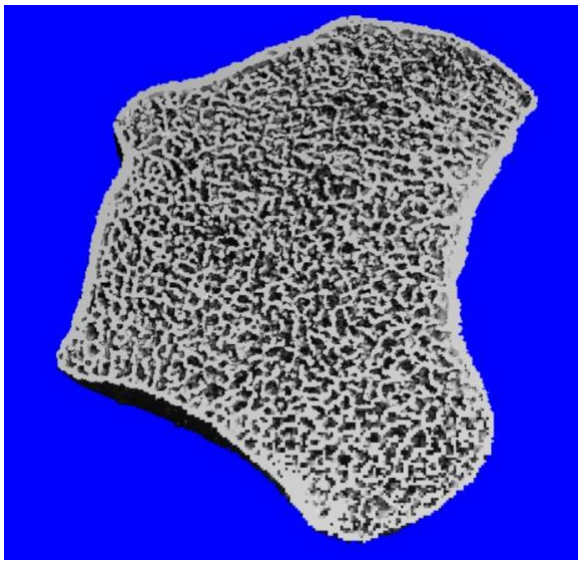
In the Academic Unit of Bone Metabolism, the long-term coefficient of variation for lumbar spine DXA scans is 1.6% for aBMD and 2.0% for total hip for postmenopausal women with normal BMI (24). The scan technician is certified by the ISCD.

Hight resolution peripheral quantitative computed tomography

Principles

High resolution peripheral quantitative computed tomography (HR-pQCT) provides high resolution images of the distal appendicular skeleton, using low radiation dose. The first generation XtremeCT device and the SCANCO Image Processing Language (IPL, version 5.08-B) (SCANCO Medical AG: Brüttisellen, Switzerland) was used to quantify vBMD and bone microstructure at the radius and tibia. The device includes a rotating x-ray tube and a static 2D detector array. The X-rays generated by the rotating x-ray tube pass through a section of the limb being scanned and is detected by a static 2D detector array. An attenuation profile is detected and the spatial distribution of this attenuation is computed onto a blank matrix to generate an image (68). The series of parallel consecutive image slices is computed into a high-resolution 3D image (Figure 2-11).

A



B

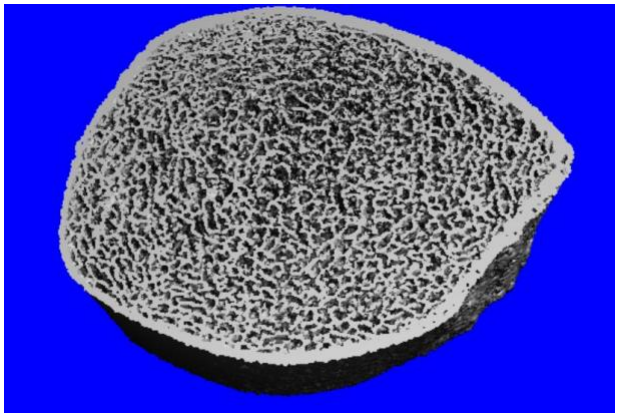


Figure 2-11 HR-pQCT images 3D images A radius, B tibia

In order to calculate volumetric BMD, a pre-calibration using a phantom is required. A phantom with five hydroxyapatite resins compartments was used to calibrate the scanner. The phantom has compartments with known progressively increasing densities, from 0mgHA/cm^3 (equivalent to soft tissue with no mineral content) to 800 mgHA/cm^3 (Figure 2-12). The phantom was scanned and the attenuation of the image slices were calculated. This pre-calibration allows the conversion of attenuation values into volumetric BMD values in mgHA/cm^3.

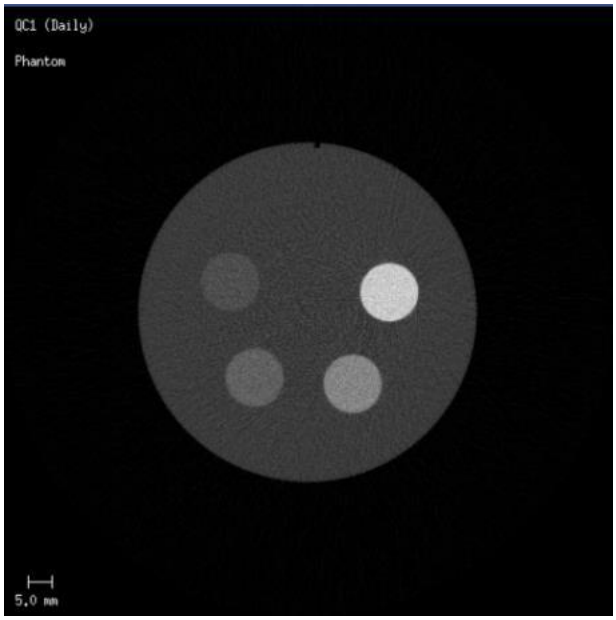


Figure 2-12 HR-pQCT calibration phantom image

Procedure

The non-dominant limb was scanned, unless the participants reported a previous fracture on the non-dominant limb. The high-resolution mode (image matrix =1536x1536) was used with

a source potential of 60kVp. The tube current was 900mA and the integration time 100ms. A reference line was placed in the site of interest to place the first scan line. From there, 110 slices were acquired resulting in a stack height of 9.8mm.

The technician asked the participant to remain motionless during the image acquisition. Upon the completion of each scan, image quality of a single slice was evaluated using the visual grading system reported by Engelke et al (69):

Grade 1 = Perfect: No noticeable artefacts.

Grade 2 = Slight artefact: small streaking.

Grade 3 = Pronounced artefact: large streaking, particularly near the cortex.

Grade 4 = Unacceptable artefacts: discontinuity at the cortex.

Images graded ≥ 3 were repeated. Images graded 4 were not included in the evaluation.

Distal radius

Participants were asked to sit in a particular chair, that allows required positioning adjustments. A forearm cast was used to position the arm. The hand and lower arm were placed into the forearm cast and an arm pad was used to stabilise the arm. The arm was then placed into the device and secured (Figure 2-13). The procedure was repeated for each radial scan.

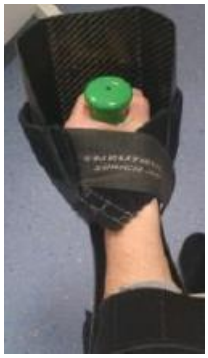
A  B  C 

Figure 2-13 Distal radius HR-pQCT scan positioning

A participant's positioning; B arm cast; C participant's arm positioned in the cast.

Standard site

Following the arm positioning, a scout scan was performed to determine the measurement using standard procedures. In the scout image, the notch on the articular surface of the distal

radius was identified. The reference line was placed on that site. The measurement started 9.5 mm from the reference line (Figure 2-14). Participants were requested to remain motionless during the scan. After the scanning acquisition, the operator visually inspected random images to assess quality. If important motion artefact was detected, the scan was repeated once (69).

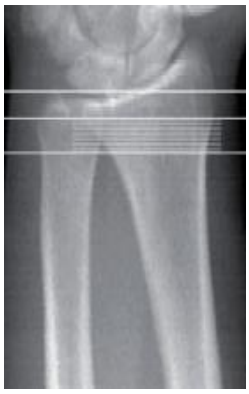


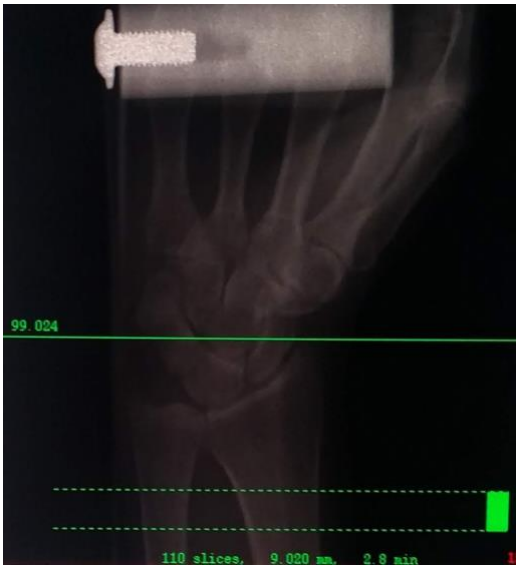
Figure 2-14 Positioning of the reference line on the radius scout scan for standard measure

14% site

The forearm length was measured following standard procedures. In summary, with the participant's elbow flexed and the back of his or her hand facing the technician, the length from the olecranon to the ulnar styloid process was measured. The total length was recorded and 14% of the distance was calculated.

As the 14% site is not a usual site measured by HR-pQCT the control file was edited prior to each measurement. Each participant's radius 14% length was inserted in the relative position to scout view reference line, ensuring that the first slice of the measurement was acquired 1 mm proximal to the 14% site. The scan was then pre-calibrated before the participant's arm was positioned within the scanner.

The participant was then positioned and the scout view scan performed. On the scout view, the reference line was placed. A solid green line was positioned on the distal end of the radius. The dotted green lines indicate the measurement region (Figure 2-15). Following the reference line positioning the main scan was performed. As in the standard site, the operator assessed the quality of the scans after each procedure. Scans could be repeated once.

A 

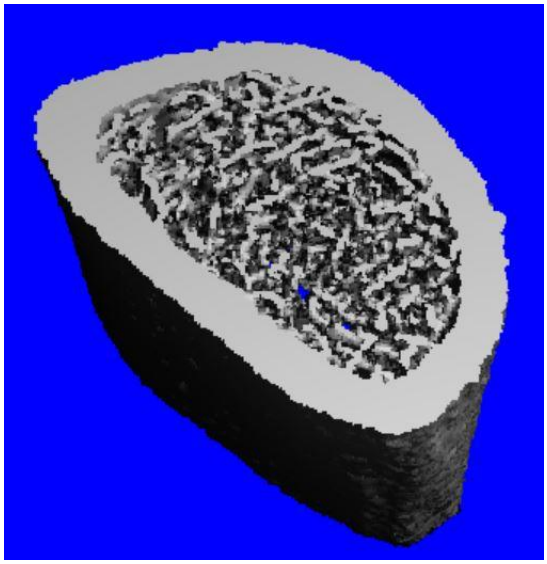
B 

Figure 2-15 A Positioning of the reference line on the radius scout scan for 14% site measure B 3D-image radius 14%

Distal tibia

For the tibia scanning, the foot and lower leg was placed into the tibia cast with the participant sited in the scanning chair. With the leg rested on the leg support, the participant was positioned. The chair was adjusted so that the leg height was the same height as the gantry (Figure 2-16).

A 
B 
C 

Figure 2-16 Distal tibia HR-pQCT scan positioning

A participant's positioning; B leg and foot cast; C participant's leg positioned in the cast.

The scout scan was performed and the reference line placed at the endplate of the distal tibia (Figure 2-17). The first slice measured was placed 22.5 mm from the reference line, following standard protocols.

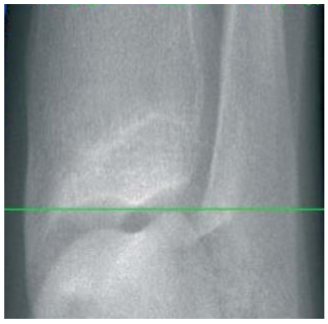


Figure 2-17 Positioning of the reference line on the tibia scout scan for standard measure

14% site

The tibia length was measured following standard procedures. In summary, with the participant's sited, foot placed flat on the floor and the knee bent to form a 90 degrees angle, the distance from the most prominent point on the lateral malleolus to the tibial lateral condyle was measured and recorded. The 14% of that distance was then calculated.

Similarly to the procedure at the radius, the control file was edited prior to each measurement. Each participant's tibia 14% length was inserted in the relative position to scout view reference line, ensuring that the first slice of the measurement was acquired 1 mm proximal to the 14% site. The scan was then pre-calibrated before the participant's leg was positioned within the scanner.

The participant was then positioned and the scout view scan performed. On the scout view, the reference line was then placed. A solid green line was positioned on the distal end of the tibia. The dotted green lines indicate the measurement region (Figure 2-18). Following the reference line positioning the main scan was performed. As in the standard site, the operator assessed the quality of the scans after each procedure. Scans could be repeated once.

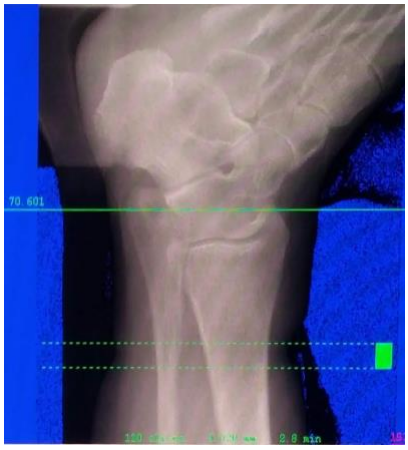
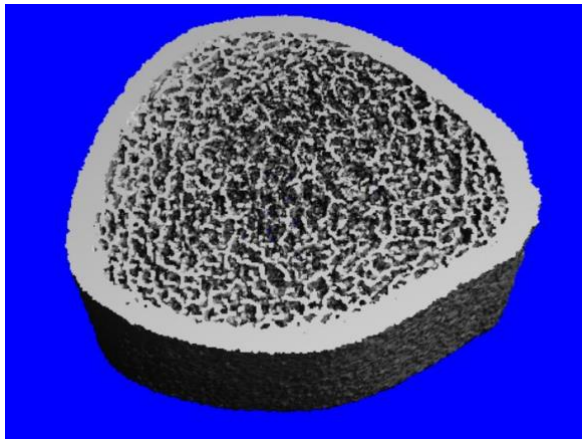
A  B 

Figure 2-18 A Positioning of the reference line on the tibia scout scan for 14% site measure; B 3D-image Tibia 14%

HR-pQCT outcomes

Table 2.3 HR-pQCT outcomes

Measurement	Abbrev	Unit	Source of measurement
Total Area	Tt.Ar	mm^2	Total cross-sectional area inside the periosteal envelope
Cortical area	Ct.Ar	mm^2	Cortical bone area.cortical volume (Ct.V) / (number of slices x slice thickness)
Trabecular area	Tb.Ar	mm^2	Mean surface area of the trabecular compartment
Volumetric total BMD	vBMD	mg HA/cm^3	Total mineral mass divided by the total bone volume
Trabecular vBMD	Tb.vBMD	mg HA/cm^3	Trabecular mineral mass divided by the volume inside the cortical bone
Meta trabecular density	Dmeta	mgHA/cm^3	Density of inner 40% of trabecular region
Inner trabecular density	Dinn	mgHA/cm^3	Density of outer 60% of trabecular region

Measurement	Abbrev	Unit	Source of measurement
Meta/Inn trabecular density		-	Meta trabecular density divided by inner trabecular density
Trabecular thickness	Tb.Th	μm	Mean thickness of trabeculae within the trabecular compartment
Trabecular number	Tb.N	mm^{-1}	Mean number of trabeculae per mm within the trabecular compartment
Trabecular separation	Tb.Sp	μm	Mean distance between trabeculae within the trabecular compartment
Trabecular inhomogeneity			SD of the intra-individual distribution of trabecular separation
Trabecular bone volume fraction	BV/TV	%	Derived by dividing Tb.vBMD by an assumed 100% mineralisation of 1200 mgHA/cm3
Connectivity Density	Conn.D	$1/mm^3$	A measure of the degree of connectivity of trabeculae normalized by TV
Cortical vBMD	Ct.vBMD	mg HA/cm^3	Cortical mineral mass divided by the cortical volume
Cortical thickness	Ct.Th	μm	Mean thickness between the periosteal and endosteal surfaces
Cortical porosity	Ct.Po	%	Cortical porosity: In a given cortical region, the volume of pores (Po.V, mm3) / total volume of cortical bone compartment (Ct.V, mm3)
SD of mean cortical pore diameter	Ct.Po.Dm.SD	mm	Standard deviation of the mean cortical pore diameter
Cortical tissue mineral density	Ct.TMD	mg/cm3	TMD is calculated from the average attenuation

Measurement	Abbrev	Unit	Source of measurement
			value of the bone tissue only and does not include attenuation values from non-bone voxels
Cortical pore volume	Ct.PoV	mm^3	Total pore volume
Cortical perimeter	Ct.Pm	mm	Cortical periosteal perimeter
Periosteal perimeter	Ps.Pm	mm	Periosteal perimeter
Endosteal perimeter	Ec.Pm	mm	Endocortical perimeter
Cortical area fraction (calculated) (Ct.Ar/Tt.Ar)			Cortical area fraction
Cortical area fraction (calculated) (Ct.Ar/Tt.Ar)*100		%	Cortical area fraction

Adapted from (70)

HR-pQCT strengths and limitations

Strengths

HR-pQCT produces high resolution images of peripheral sites, using low radiation effective dose. The high-resolution images allow the distinction between cortical and trabecular bone, enabling the investigation of bone microarchitecture (71). DXA does not provide the same level of details. The peripheral sites are less affected by soft tissue confounding and do not result in the exposure of extensive areas to radiation (71). These are common disadvantages of axial quantitative computer tomography. The procedure is quick and the total effective radiation effective dose is 3μSv, similar to one day background radiation exposure (71). HR-pQCT provides microarchitectural assessment of the tibia and the radius, sites commonly affected by fractures.

Limitations

HR-pQCT image acquisition and analysis has several limitations. The scanner has a narrow gantry and a restricted scanning field. This could restrict the scanning of less distal areas.

The voxel size of the scanner (82μm) is close to the average thickness of the trabecular structure and some of the parameters, e.g. trabecular thickness, are derived rather than directly measured (71). In addition, both cortical and trabecular analysis is dependent on resolution (72). Furthermore, the voxel size limits the detection of pores in the cortex to the bigger ones. Noteworthily, the voxel size is not equivalent to true spatial resolution (72).

Furthermore, the segmentation of images is limited. One of the main benefits of HR-pQCT is the assessment of trabecular and cortical compartments, however, there is no clear defining border between the two compartments. Instead there is a transitional zone. The proper identification of the cortex is a challenge, especially when the cortex is thin or highly porous (71).

Finally, HR-pQCT assesses peripheral sites. These sites are commonly affected by fractures; however, these are not the most serious osteoporotic fractures. How well peripheral measurements reflect axial properties is unknown. Studies that compared peripheral and central assessment by DXA, HR-pQCT and cQCT reported that distal radius and tibia reflect the stiffness of lumbar spine and proximal femur. Moderate to strong correlations (r.0.56 – 0.70) have been reported between peripheral and axial sites stiffness (73).

Micro Finite Element analysis

The Micro Finite Element (μFE) software was used to determine bone strength from the HR-pQCT data (version 1.13; FE-solver included in the Image Processing Language, Scanco Medical AG, Zurich, Switzerland). The software uses mathematical modeling to simulate strength-determining biomechanical tests using trabecular and cortical microarchitecture features. The software is fully automated and validated and provides an *in vivo* assessment of bone strength (74). The method provides a reliable estimate of bone strength however, it cannot account for all bone material features, as inhomogeneity of mineralization, or all variations on the biomechanics of falls.

Table 2.4 list the outcomes of μFE analysis.

Table 2.4 FE analysis outcomes from HR-pQCT scanning

Outcome	**Abreviation**	**Unit**	**Definition**
Stiffness	Stiffness	kN/mm	Resistance to deformation when applying a load; total reaction force divided by displacement
Estimated failure load	Est.Fail.Load	kN	Maximum load the bone can bear before fracture; when 2% of the bone is strained beyond 3500 μstrain
Percent trabecular proximal load	%TPL	%	The distribution of the load between the cortical and trabecular compartments
Percent trabecular distal load	%TDL	%	The distribution of the load between the cortical and trabecular compartments
Percent cortical proximal load	%CPL	%	The distribution of the load between the cortical and trabecular compartments
Percent cortical distal load	%CDL	%	The distribution of the load between the cortical and trabecular compartments
Mean trabecular Von Mises stress	Tb.VM	MPa	Indicates whether combined stresses in the x, y and z directions in the trabeculae will cause failure
Mean cortical Von Mises stress	C.VM	MPa	Indicates whether combined stresses in the x, y and z directions in the cortex will cause failure

Chapter 3

The Risk of Hip and Non-vertebral Fractures in Diabetes: A Systematic Review and Meta-Analysis update

Chapter 3

The Risk of Hip and Non-vertebral Fractures in Diabetes: A Systematic Review and Meta-Analysis update

This chapter was published as a paper on Bone;
Vilaca T, Schini M, Harnan S, et al. The risk of hip and non-vertebral fractures in type 1 and type 2 diabetes: A systematic review and meta-analysis update [published online ahead of print, 2020 May 29]. *Bone*. 2020;137:115457. doi:10.1016/j.bone.2020.115457

Introduction

Diabetes is a growing epidemic worldwide. Data from the World Health Organization (WHO) reported that the number of people with diabetes has risen from 108 million people in 1980 to 422 million people in 2014 and the global prevalence has increased from 4.7% to 8.5%. In 2016, 1.6 million deaths were directly caused by diabetes. Almost half of the deaths attributable to diabetes occur before the age of 70 years (75). In the United Kingdom (UK), 7.7% of the population has diabetes and 10% of the NHS budget from England and Wales is spent on diabetes (76, 77). Estimates suggest that £14 billion is spent every year on treating diabetes and its complications (77). In the United States, 9.1% of the population has diabetes and the estimated economic cost in 2012 was $245 billion (78, 79).

Osteoporosis is also a public health concern. Estimates suggest that osteoporosis causes almost 9 million fractures annually, more than half of these in Europe and the Americas (80). From this pool, 1.6 million fractures affect the hip, 1.7 million the forearm and 1.4 million are clinical vertebral fractures (80). Hip fractures are associated with the greatest morbidity and mortality. Estimates suggest that up to 20% of patients die in the first year after a hip fracture and less than half regain the previous level of function (80).Mortality after a hip fractures is higher in patients with diabetes than in people without diabetes (81). Besides the high morbidity and mortality, huge economic costs are also involved. In 2017, the fracture-related costs in the UK was estimated at £5.25 billion and it is expected to increase 30% by 2030 (82). In the US, estimates suggest a yearly $20 billion cost with fractures (83).

Some previous reviews have reported an increase in the risk of hip fractures in both T1D and T2D and an increase in the risk of vertebral fractures in T1D (10, 13, 14, 84-86). In addition,

a greater increase in the risk of hip fractures in T1D than in T2D has also been reported (10, 84, 85). Although a number of reviews have assessed the risk of fractures in diabetes, no recent review has addressed the risk of non-vertebral fractures in this population. Hip, vertebral and non-vertebral fractures are common sites used in clinical trials to assess drug efficacy (87). Among these sites, it is known that data on hip fractures is reliable. As all hip fractures are treated by surgery, they are reliably captured in hospital records. Conversely, vertebral fractures are commonly undiagnosed and need to be identified on spinal imaging. This makes the registry data unreliable and inadequate to assess the risk of vertebral fractures. This inaccuracy could compromise the analyses not only of vertebral fracture as a site but also the all fractures analysis as vertebral fractures are included. In this scenario, the non-vertebral fractures risk emerges as an alternative for an overall picture of the risk of fractures in diabetes to be assessed in systematic reviews. Furthermore, the current meta-analyses have not fully explored the effect of other features in the risk of fractures in diabetes. Some studies have investigated the effect of gender (10, 85, 86, 88), diabetes type (10, 84-86), geographical location (10, 13, 85) and study design (10, 13, 86, 89). However, none of the previous studies has investigated the effect of age, BMI, diabetes duration, insulin use and the presence of complications. We conducted a recent meta-analysis on the risk of fractures in chronic kidney disease and found a greater increase in younger populations, suggesting that the pattern of fractures might be different in chronic diseases {Vilaca Tatiane , 2019, The Risk of Hip and Non-Vertebral Fractures in Chronic Kidney Disease: A Systematic Review and Meta-Analysis}. In addition, there is evidence that obesity has a protective effect in the skeleton (67). A few studies suggested that longer diabetes duration (90, 91), insulin use (92, 93) and the presence of complications (64, 94) were associated with a greater increase in the risk of fractures in diabetes. These features have not been explored in previous meta-analyses.

The aim of this systematic review and meta-analysis was to assess the risk of hip and non-vertebral fractures in adults with diabetes compared to adults without diabetes in observational studies. We also assessed if gender, age, BMI and diabetes-related features such as DM type, duration, insulin use and the presence of complications affect this risk.

Methods

This review complies with key principles from the Cochrane Handbook and the Centre for Reviews Dissemination Handbook (56, 95). This report followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (96). The protocol was registered in PROSPERO. The record number is CRD42018090378.

Searches

The search strategy was to identify a published systematic review that we could then update. This review was part of a broader project conducted in collaboration with the School of Health and Related Research (ScHARR) that investigated the risk of fractures in chronic diseases following a mini-review previously published (97). The aim was to investigate the risk of hip and non-vertebral fractures in diabetes, Chronic Kidney Disease and Parkinson's Disease. Where previous reviews were identified, their quality was assessed, and the best review was selected and updated. We conducted searches to identify systematic reviews, followed by primary studies searches. The initial searches were conducted simultaneously for diabetes, CKD and Parkinson's Disease. One review on the risk of fractures in diabetes was selected to be updated and the primary studies research was conducted from the date of the selected review search, June 2006. The full search strategies are described in Appendix 1. In summary, we combined terms for fractures and diabetes mellitus and related synonyms including free and thesaurus terms. We used Boolean operators and database-specific syntax.

For both searches, the following databases were searched: Ovid MEDLINE(R); Ovid MEDLINE(R) Epub Ahead of Print; Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations; Ovid MEDLINE(R) Daily Update; Embase via Ovid. For the systematic review searches, the following additional databases were searched: Cochrane Database of Systematic Reviews; Database of Abstracts of Reviews of Effect via The Cochrane Library; and the Health Technology Assessment Database via The Cochrane Library. For the primary study searches, the Cochrane Central Register of Controlled Trials was also searched.

The reference lists of key existing reviews were searched for additional primary studies (13, 84, 86, 98, 99) and experts in the field were consulted for additional relevant studies.

Study selection

Search results were uploaded to Endnote and the duplicates were removed. For both the previous reviews and primary studies searches, one reviewer excluded clearly irrelevant records on the basis of their title and abstracts against the inclusion and exclusion criteria. A second reviewer independently sifted a 10% sample and the kappa statistic for the agreement was calculated. The full test sift was conducted by one reviewer in the reviews search and independently by two reviewers in the primary study search. Disagreements were resolved through discussion or involvement of a third reviewer. Studies that addressed some or all of the same population were included if they reported different aspects of that population that could be used in subgroup analyses. Potential small overlaps due to nationwide surveys or cohorts that recruited in the same region were considered non-relevant. Table 3.1 describes the inclusion and exclusion criteria.

Table 3.1 Inclusion and exclusion criteria for the review on the risk of fractures in diabetes

	Inclusion criteria	**Exclusion criteria**
Population	Adult (aged 18 years and above) DM patients who have a diagnosis of type 1 or type 2 DM.	Studies lacking a clear definition/diagnosis of DM Studies with heterogeneous populations without data on/unclear data on DM patients (e.g. where the population is not clearly defined) Studies where diagnosis of DM is made following fracture Studies without a clear sequence between diagnosis of DM and fracture
Comparison	Adults (aged 18 years and above) who do not have DM	Studies lacking a clear description of the comparison group.
Outcome	Hip and non-vertebral fractures (i.e. all fracture sites	Data only on the occurrence of spine/vertebral fracture

	Inclusion criteria	Exclusion criteria
	excluding spine/vertebral), adjusted for age and gender*	Studies reporting predicted fracture risk based on an algorithm or risk tool Studies with unclear/incomplete/missing data Studies where risk estimates were not adjusted for age and gender*.
Study design	<u>Review of Systematic reviews</u>: Systematic reviews of observational studies on risk of hip or non-vertebral fractures <u>Primary study review:</u> Observational studies on risk of hip or non-vertebral fractures	Studies not published in full text in English language Narrative reviews, letters, editorials, commentaries, conference abstracts, animal studies, biological studies will also be excluded.

DM, diabetes mellitus

*** The criteria relating to adjustments for age and gender was an amendment to the protocol, made after full text sifting had commenced but before data extraction commenced. Age adjustment was considered important, as fracture risk is affected by age.**

Data extraction

We used a standardised piloted data extraction form to extract the data from the full text of all papers, including the ones from the selected review (Data extraction form in appendix 2). Data were extracted by one reviewer and checked by another reviewer and disagreements were resolved through discussion.

Quality assessment

The Newcastle Ottawa Scales (NOS) was used to assess the quality of primary studies. The tool assesses the selection and comparability of the study groups, and the ascertainment of exposure (for case-control studies) or outcome of interest (for cohort studies). Stars are awarded to a maximum of nine. The scoring was adapted for the review question. We considered age and gender the most important factors to be controlled for and a follow-up of 80% or greater unlikely to introduce bias. Each study was assessed by one reviewer and checked by a second and disagreements were resolved through discussion or involvement of a third reviewer. The adapted NOS scoring template is provided in appendix 3.

Meta-analysis methods

Some studies reported the risk estimates in several categories, such as gender, age groups and diabetes type. Studies that reported more than two risk estimate for a given group in the subgroup analyses were summarised using the random-effects model. For the non-vertebral fracture analyses, studies that reported the risk of fractures for two or more sites were summarised using the random effects model. Subgroup analyses for gender, age, BMI and diabetes-related features such as DM type, duration, insulin use and the presence of complications were anticipated in the protocol and performed when enough data was available. An exploratory analysis by geographical location was added. Subgroup RR were considered significantly different if there was no overlap in the 95% CI. However, if there was a small overlap we used the ratio of relative risk (RRR) and the 95% CI to compare the risk. If the 95% confidence interval did not include the unit, the subgroups RR were considered statistically different (100).

Some studies described the same population but reported the risk for different groups. These studies were included in different subgroup analysis, but a given population/cohort was not included twice in the same analysis. For the overall analysis the most comprehensive data was included. For subgroup analysis, the study that addressed that specific feature of interest was included. Conversely, the studies that did not report the risk for that specific subgroup analysed were not included, i.e. in the analysis by gender, studies that reported one risk estimate including female and male were not included. We used the random-effects model to pool the studies.

Heterogeneity, when high, was explored by subgroup analysis, sensitivity analysis and meta-regression. Subgroup analyses were performed when enough data was available. We performed a sensitivity analysis excluding one study at a time. We also excluded the case-control studies and the studies that scored less than seven in the quality assessment. To explore the effect of the several risk estimates reported, such as hazard ratio, relative risk, …, we performed sensitivity analysis excluding the studies that reported each risk estimate. For example, we performed sensitive analysis excluding all the studies that reported hazard ratios. In the subgroup analysis by geographic location, in contrast with other subgroups, the Australian subgroup showed a wide confidence interval; we performed sensitivity analysis excluding these studies. In the hip fracture analysis, meta-regression was performed to assess how much of the variation observed was due to diabetes type or age group (< 65 years vs ≥ 65 years) individually and combined.

We used the visual analysis of funnel plots to assess publication bias. When visual analysis suggested publication bias, additional tests such as Begg's and Eggers where used (101). If the additional tests confirmed the small studies effect the Trim and Fill method was applied (102).

Results

Study selection

The search for systematic reviews identified 452 unique records. The assessment of the title and abstract excluded 388 records. From the remaining 64 records, one systematic review was selected (10). The kappa statistic for the agreement between reviewers about studies selection was perfect (1.00 95%CI 1.0, 1.0).

The searches for primary studies identified 3081 records, including the 81 identified in March 2019, in the search update. Duplicates were excluded resulting in 1794 unique papers. Searches in the reference lists of relevant papers and contact with experts in the field retrieved a further 32 records. Hence, 1826 records underwent the title and abstract sifting. Of these, 1609 were considered irrelevant and 217 records underwent full-text assessment against inclusion and exclusion criteria. A further 168 were excluded (list of reasons in appendix 4), resulting in 49 studies that met the inclusion and exclusion criteria. Of these, 48 were included in the meta-analyses, 42 in the hip fractures analysis (15, 64, 90-93, 103-138)

and 17 in the analysis of non-vertebral fractures (90, 92, 93, 103, 113, 115-117, 119, 132, 134, 137, 139-143). The search process is described in the PRISMA diagram (Figure 3-1)

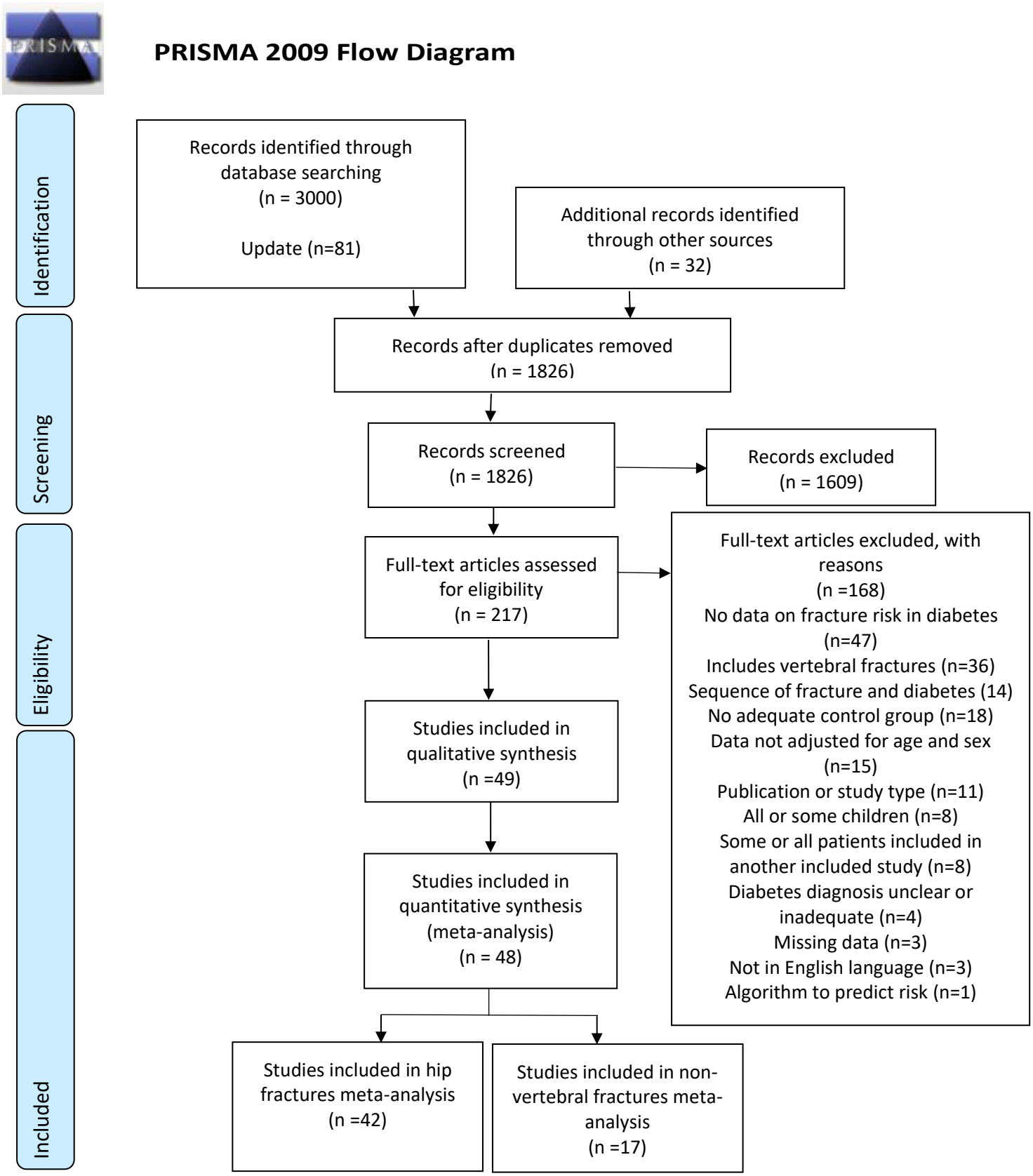


Figure 3-1 Search process diagram

Hip fracture study characteristics

Table 3.2 summarises the study characteristics. Forty-three studies reported data on hip fracture risk in people with diabetes compared to people without diabetes (15, 64, 91-93, 103-138, 144). Eleven analysed overlapping populations but reported subgroup data relevant to our subgroup analyses (90, 105, 106, 113, 114, 120-122, 126, 127, 144). One study reported the RR according to metabolic control and was not included in calculations (144). Forty studies were cohorts (22 prospective(90, 91, 93, 103, 107, 108, 110-112, 115, 116, 118, 119, 123, 128-130, 133, 134, 136, 144) and 18 retrospective (15, 64, 104-106, 113, 114, 117, 120-122, 124-127, 132, 137, 138)) and three studies were case-control studies (109, 131, 135). The study size varied from 238 (135) to 3,861,874 participants (15) and they were published from 1993 (128) to 2019 (123). Nineteen studies were from North America; five from Canada (120-124) and others from the USA (64, 91, 92, 104, 109, 119, 125, 129-131, 133, 134, 136, 137). Sixteen studies were from Europe; three from Norway (103, 108, 128), two from the Netherlands (90, 144), one from Austria (107), three from the United Kingdom (15, 112, 138), two from Denmark (113, 114), two from Sweden (93, 115), two from Spain (126, 127), and one from Germany (132). Five studies were from Asia (Taiwan (105, 106), Korea (117), Singapore (118) and Israel (135)) and three from Australia (110, 111, 116). Two studies reported data only from T1D participants (111, 138), ten studies reported data only from T2D participants (90, 92, 107, 110, 117, 126, 127, 131, 132, 144) and the others reported data from participants of both DM types (15, 64, 91, 93, 103, 108, 112, 120, 121, 123-125, 129) or did not specify the participant's DM type (104-106, 109, 113-116, 118, 119, 122, 128, 130, 133-137). Ages varied from 20 to 100 years old. Six studies reported data just from women (91, 92, 107, 119, 122, 129) and three just from men (64, 127, 131). The other studies reported data from both genders and the percentage of women varied from 32% (115) to 94% (135). One study reported data from two cohorts separately, the Women's Health Initiative (WHI) and the North Carolina Established Populations for Epidemiologic Studies of the Elderly EPESE (119). However, the WHI cohort was more comprehensively described by Robbins et al (133) and Bonds et al (139) and only the data from the EPESE cohort was used from Lee, 2015 (119). Not all studies reported the population ethnicity. Studies from Asia were included (105, 106, 117, 118, 135) and some studies from North America included blacks and Hispanics (64, 104, 119, 125, 130, 133, 134, 136, 137) , but the majority of data reported addressed white populations.

Hip fracture - Quality assessment

We report a summary of the authors' judgement in Table 3.3 (cohort) and Table 3.4 (case-control). The full description of the criteria and the author's judgement with reason is described in appendix 4. Overall the quality of the studies was good as most scored higher than seven, which is considered high quality. The main criterion not addressed by the studies was the adequacy of follow up; twenty-three studies did not score in this criterion, mainly due to the lack of information (15, 64, 90, 92, 103, 104, 107, 108, 110, 111, 116, 119, 123, 124, 129, 130, 132-134, 136-138, 144). Another criterion in which the studies did not score was representativeness. Volunteers are usually healthier than people who do not volunteer in studies, so they were not considered representative of the community (145). One cohort study scored five (129). This study is a prospective cohort that surveyed post-menopausal women by post for 11 years. Consequently, this study did not score in representativeness (volunteers), ascertainment of exposure and assessment of the outcome (both self-reported) and adequacy of follow-up (bellow 80% in the last survey). Five studies scored six; one case-control and four cohort studies (91, 110, 111, 133, 135). All these cohort studies scored poorly in representativeness, for recruiting volunteers and in the ascertainment of exposure (not reported (110, 111) or self-reported (91, 133)). Three of them lost scores in the adequacy of follow-up (110, 111, 133) and Janghorbani et al lost scores in the assessment of outcome as fractures were self-reported (91). The case-control study also lost scores in representativeness, ascertainment of exposure and adequacy of follow-up. In this study, controls were first incident fractures while cases were any fractures and data on exposure and follow-up were unclear (135).

Table 3.2 Hip fractures study characteristics

Study, year	Study design	Country	Cohort name	DM type	Age (y)	Follow-up y (SD)	Pop total / DM	Fract	Ethnicity (%)	Gender (% female)	Risk estimate group	Risk estimate
Ahmed, 2006	Cohort[1]	Norway	The Tromsø study	Both	25-98	6	27,159/ 455	1249	NR	52	Calculated overall	3.9 (1.19-12.8)[3]
Berry, 2017	Cohort[2]	USA	FRAiL	NS	65-113	1.8	419,668/ 119,490	14,553	White 83% Black 13% Hispanic 2% Asian 1% Native American 0.4%, Others/ Unknown 0.8%	71	Overall	1.09 (1.05-1.13)[4]
Chen, 2008	Cohort[2]	Taiwan	Taiwan NHI	NS	> 35	6	969,821/ 484787	20220	NR	53	Male	1.28 (1.21–1.34)[5]
											Female	1.72 (1.66–1.78)[4]
Lai, 2015	Cohort[2]	Taiwan	Taiwan NHI	NS	≥65	5	81,245/ 16249	4005	NR	48	DM < 5y	1.20(1.14, 1.26)[6]
											DM ≥ 5y	1.37(1.28, 1.46)[6]
de Liefde, 2005	Cohort[1]	Nether-lands	Rotterdam Study	T2D	≥65	5.2 (3.6)	6,655/ 792	771	NR	59	Overall	1.18 (0.76–1.83)[7]
Oei, 2013	Cohort[1]	Nether-lands	Rotterdam Study	T2D	≥55	12.2 (4.2)	4,135/ 420	1068	NR	59	ACD	1.15 (0.68-1.94)[8]
										59	ICD	0.96 (0.52-1.75)[8]
Dobnig, 2006	Cohort[1]	Austria	Austrian nursing homes	T2D	>70	2	1,664/ 583	110	White	100	Overall	0.90 (0.60 –1.34)[9]
Forsen, 1999	Cohort[1]	Norway	Nord-Trùndelag Health Survey	Both	≥50	9	35,444/ 1850	1643	NR (Norwegian)	52	Calculated overall	1.23 (0.95-1.59)[10]

Study, year	Study design	Country	Cohort name	DM type	Age (y)	Follow-up y (SD)	Pop total / DM	Fract	Ethnicity (%)	Gender (% female)	Risk estimate group	Risk estimate
Gerber, 2013	Case-control	USA	Olsmted County, Minnesota	NS	>50	1985-2006	3,808/ 559	1904	White	76	By period	1985-1999 1.03 (0.83-1.31)[11] 2000-2006 1.77 (1.33-2.35)[11]
Hamilton, 2017b	Cohort[1]	Australia	Fremantle Diabetes Study phase I	T1D	NR	14.5 (5.8)	605/ 121	14	NR	40	Overall	7.11 (2.45–20.64)[4]
Hamilton, 2017a	Cohort[1]	Australia	Fremantle Diabetes Study phase I	T2D	NR	12.9 (6.1)	6,450/ 1291	424	White 77.5% Non-European 12.5%	51	Overall	1.34 (1.06–1.69)[4]
Hippisley-Cox, 2012	Cohort[1]	UK	v32 of the QResearch database	Both	30-100	NR	3,142,673/ 97,537	23810	White or not recorded 95.3%. Indian 0.9% Pakistani 0.5% Bangladeshi 0.3% Other Asian 0.5% Caribbean 0.5% Black African 0.8% Chinese 0.2% Other 0.9%	51	Calculated overall	2.48 (1.65-3.72)[12]
Holm, 2018	Cohort[2]	Denmark	Danish National registries	NS	NR	NR	6,285/ 229	NR	NR	NR	T2D female	1.31 (1.02-3.31)[13]
Jorgensen, 2014	Cohort[2]	Denmark	Danish National registries	NS	≥65	NR	1,276,891/ NR	89150	NR	58	Overall	1.12 (1.09-1.14)[14]
Holmberg, 2006	Cohort[1]	Sweden	Malmö Preventive Project	NS	NR	F 11 M 16	33,346/ NR	3915	NR	32	Female	4.07 (1.79-9.26)[3]
											Male	7.75 (4.37- 13.7)[3]

Study, year	Study design	Country	Cohort name	DM type	Age (y)	Follow-up y (SD)	Pop total / DM	Fract	Ethnicity (%)	Gender (% female)	Risk estimate group	Risk estimate
Hothersall, 2014	Cohort[2]	Scotland	SCI-DC	Both	≥20	NR	3,861,874/ 201,874	13,259	NR	NR	Calculated overall	1.76 (1.3-2.39)[15]
Ivers, 2001	Cohort[1]	Australia	The Blue Montains Eye Study	NS	≥ 49	5	3,654/ 216	251	NR	57		0.6 (0.2–2.2)[3]
Janghorbani, 2006	Cohort[1]	USA	NHS	Both	30-55	18 (T1D) -20 Non-DM	109,983/ 8,640	1398	White 98%	100	T1D	7.1 (4.4–11.4)[16]
											T2D	1.7 (1.4–2.0)[16]
Kim, 2017	Cohort[2]	Korea	KNHIS	T2D	≥50	6	51,330/ 17,110	1,816	NR, Korean	54	Female	2.11 (1.71–2.60)[16]
											Male	1.81 (1.30–2.52)[16]
Koh, 2010	Cohort[1]	Singapore	Singapore Chinese Health Study	NS	45–74	12.2, (3.3)	63,154/ 5,668	1213	NR, Chinese	DM 57 Non-DM 56	Overall	2.00 (1.73–2.31)[17]
Lee, 2015 (EPESE)	Cohort[1]	USA	EPESE	NS	≥ 65	6.5	2,704/ 566	hip 173	Blacks 54.5% White 45% Others 0.5%	100	Overall	1.27 (0.80–2.02)[18]
	Cohort[2]	USA	VHA	Both (98 % T2D)	65-99	NR	2,798,309/ 900,402	11,176	White 71.5% Black 8.4% Other 3.9% Unknown 16.1%	0	Overall	1.21 (1.19–1.23)[19]
Leslie, 2007	Cohort[2]	Canada Manitoba	POPULIS MCHP	Both	≥20	NR	318,776/ 82,094	17,342	NR but Aborigines 7.2% controls, 10.7 % DM	50	Calculated overall	1.1 (0.59-1.51)[20]
Leslie, 2014	Cohort[2]	Canada Manitoba	Registry of DXA, Manitoba, Canada	Both	≥40	6	62,413/ 6,455	1108	White 97.8%	Controls 92 DM 86	<60	4.67 (2.76–7.89)[21]
											60-69	2.68 (1.77–4.04)[21]
											70-79	1.57 (1.20–2.04)[21]
											≥80	1.42 (1.01– 1.99)[21]

Study, year	Study design	Country	Cohort name	DM type	Age (y)	Follow-up y (SD)	Pop total / DM	Fract	Ethnicity (%)	Gender (% female)	Risk estimate group	Risk estimate
Majumdar, 2016	Cohort[2]	Canada Manitoba	The Manitoba BMD Cohort	NS	≥40	7	57,938/ 8,840	1388	NR	100	Female	1.32 (1.03–1.69)[22]
Li, 2019	Cohort[1]	Canada	CaMos	Both (98 % T2D)	≥ 25	9.2 (4.5)	3,149/ 138	67	NR	70	Overall	2.60 (1.04–6.55)[23]
Lipscombe, 2007	Cohort[2]	Canada	Ontario database	Both (90 % T2D)	≥66	6.1	598,812/ 197,412	22267	NR,	49	Female	1.11 (1.08–1.15)[24]
											Male	1.18 (1.12–1.24)[24]
Looker, 2016	Cohort[2]	USA	NHANESIIIN HANES 1999-2004	Both (3% T1D)	≥ 65	6.7	5,032/ 897	298	NHW 61% NHB 17% MA 17.5% Other 3.3%	49	Overall	1.35(0.82-2.22)[25]
Martinez-Laguna, 2015	Cohort[2]	Spain	SIDIAP database	T2D	NR	Median 2.63	171,931/ 58,483	1220	NR	43	Overall	1.11 (0.99-1.24)[26]
Reyes, 2014	Cohort[2]	Spain	SIDIAP database	T2D	≥65	median 2.99 (2.37–2.99)	186,171/ 36,865	1718	NR	0	Male	1.45 (1.25–1.69)[27]
Meyer, 1993	Cohort[1]	Norway	Cardiovascular screening of the National Health Screening Service	NS	35-49	10.9	52,313/ 298	212	NR	48	Female	5.81 (2.15-15.71)[4]
											Male	7.67 (2.40-24.53)[4]
Nicodemus, 2001	Cohort[1]	USA	The Iowa Women's Health Study	Both	55-69	9.5	32,089/ 1,729	490	NR	100	T1D	14.1 (5.85, 34.2)[16]
											T2D	1.75 (1.25, 2.43)[16]

Study, year	Study design	Country	Cohort name	DM type	Age (y)	Follow-up y (SD)	Pop total / DM	Fract	Ethnicity (%)	Gender (% female)	Risk estimate group	Risk estimate
Ottenbacher 2002	Cohort[1]	USA	H-EPESE	NS	≥ 65	NR	2,884/ 690	134	100% Mexican Americans	58	Overall	1.57 (1.03–2.39)[28]
Poor, 1995	Case-control	USA	Olsmted County, Minnesota	T2D	>35	1965-1989	464/ 42	232	White	0	Overall	0.9 (0.5-1.7)[16]
Rathmann, 2015	Cohort[2]	Germany	German Disease Analyzer database	T2D	NR	2.9 (SD: 3.3)	598,208/ 299,104	hip NR	NR	49	Overall	1.56 (1.45–1.67)[29]
Robbins, 2007	Cohort[1]	USA	WHI-OS	NS	50-79	7.6 (1.7)	93,676/ 38,502	1132	White 83.3% Black 8.2% Hispanic 3.9 % American Indian 0.5% Asian/Pacific Islander 2.9%	100	Overall	1.74 (1.17-2.60)[30]
Schneider, 2013	Cohort[1]	USA	ARIC Study	NS	45-64	Median 20 years	15,140/ 1,800	1078	White 74% Black 26%	55	Prevalent DM	1.76 (0.68, 4.60)[31]
											Newly diagnosed	2.99 (1.24, 7.21)[31]
Schwartz, 2001	Cohort[1]	USA	SOF	T2D	≥ 65 years	9.4 (2.4)	9,654/ 657	2624	"mainly white" (black women were excluded	100	Non-insulin user	1.49 (1.09–2.05)[16]
											Insulin user	1.26 (0.56–2.81)[16]
Segal, 2009	Case-control	Israel	NR, "Rambam Medical Center"	NS	'Elder-ly'	1	238/ 41	142	NR, Israel	Cases 76 Controls 94	Overall	3.9 (1.50–10.4)[32]
Strotmeyer, 2011	Cohort[1]	USA	CHS	NS	≥ 65	10.9 (4.6)	3,506/ 918	334	15.5% black;	58	Overall	1.05 (0.80–1.39)[33]
Taylor, 2011	Cohort[2]	USA	5% random sample of Medicare	NS	≥ 65	4.2 person-years	1,694,051/ NR	124241	White 88% Asian 1.3% African 7.8% Hispanic 1.5% Other 1.5%	58	Overall	1.01 (0.99, 1.02)[34]

Study, year	Study design	Country	Cohort name	DM type	Age (y)	Follow-up y (SD)	Pop total / DM	Fract	Ethnicity (%)	Gender (% female)	Risk estimate group	Risk estimate
Wallander, 2017	Cohort[1]	Sweden	FRAILCO	Both	≥65	median 1.3(0.6–2.3)	428,305/ 84,702	36132	NR	58	Calculated overall	1.12 (0.99-1.27)[8]
Weber,2015	Cohort[2]	UK	THIN	T1D	NR	median 4.7 (2–8.8)	334,266/ 30,394	21239	NR	44	Calculated overall	3.51 (2.7-4.55)[35]

Fract Fracture; Taiwan NHI National Health Insurance database; SCI-DC Scottish care information database collaboration; NHS Nurses' Health Study; KNHIS Korean National Health Insurance Service; EPESE North Carolina Established Populations for Epidemiologic Studies of the Elderly; VHA Veterans Health Administration; POPULIS Population Health Information System; MCHP data repository at the Manitoba Centre for Health Policy; NHW non-Hispanic white; NHB non-Hispanic black; MA Mexican American; SIDIAP Sistema d'Informació per al Desenvolupament de la Investigació en Atenció Primària; H-EPESE The Hispanic Established Population for the Epidemiologic Study of the Elderly; ARIC The Atherosclerosis Risk in Communities Study; SOF Study of Osteoporotic Fractures; CHS Cardiovascular Health Study; FRAILCO Fractures and Fall Injuries in the Elderly Cohort; THIN The Health Improvement Network

[1]Prospective [2]Retrospective
Adjustments:
[3] Age adjusted, reported by sex
[4] Age and sex
[5] Age as a continuous variable, geographic area, and urbanization status
[6] Groups were matched for sex, age and the year of diagnosis of DM
[7] Age, gender, BMI, smoking, serum creatinine, visual acuity, falling frequency, lower limb disability
[8] Age, sex, height, weight
[9] Age and weight
[10] Age, BMI and daily smoking
[11] Age and sex matched controls
[12] Ethnic origin, alcohol intake, smoking, age, BMI, medical or social factors (Asthma or chronic obstructive airways disease, any cancer, cardiovascular disease, dementia, epilepsy diagnosis or prescribed anticonvulsants , history of falls, chronic liver disease, Parkinson's disease, rheumatoid arthritis or systemic lupus erythematosus Chronic renal disease, Type 1 diabetes, Type 2 diabetes, previous fracture, endocrine disorders, gastrointestinal malabsorption, parental history of osteoporosis, any antidepressants, corticosteroids, unopposed hormone replacement therapy
[13] Adjusted for baseline age, BMI group (<20, 20–30, >30), modified Charlson index, estrogen deficiency, MOF, prevalent rheumatoid arthritis, former osteoporosis treatment, glucocorticoid use >450 prednisone eq., family fracture history, current smoking, exercise level, prevalent alcohol related diagnoses
[14] Age, gender, income, calendar year and comorbidity (ischemic heart disease, COPD, dementia, depression, diabetes, osteoporosis and stroke)
[15] Age, calendar year, SIMD, and for the overall estimate, an SIMD-age interaction
[16] Age
[17] Age at recruitment, sex (for all), year of recruitment, dialect group (Hokkien, Cantonese), level of education (no formal education, primary, secondary or higher)

[18] Age, race, BMI
[19] Adjusted for age, race/ethnicity, tobacco use, alcohol use, glucocorticoid use, rheumatoid arthritis, and BMI.
[20] Age, sex, income quintile, are of residence and ethnicity
[21] Age, sex, BMI, glucocorticoid use, rheumatoid arthritis, high alcohol use, any prior fracture, and femoral neck T-score
[22] Frax adjusted
[23] Adjusted for age, sex, and BMD femoral neck T-scores
[24] Age group chronic unstable disease; prior stroke; visual impairment; neuropathy; amputation; treatment with nitrates, statins, anticonvulsants, inhaled corticosteroids, thiazides, estrogen, and medications that increase risk of falling; and history of BMD test
[25] Age, sex and survey
[26] Age and sex matched
[27] Age, body mass index, smoking, alcohol consumption, use of oral corticosteroids, and co-morbid conditions (COPD Heart failure Chronic kidney disease, severe liver disease MLDa malignant tumour (without metastasis), metastasis, connective tissue disease, AIDS, paraplegia, dementia, peptic ulcer disease, myocardial infarction, cerebrovascular disease, peripheral vascular disease
[28] Age, gender, smoking status, BMI, and history of stroke.
[29] Age, sex, diabetologist care, depression, chronic kidney disease, peripheral vascular disease, heart failure, hyperlipidemia, obesity.
[30]Age, self-reported health, height, change in height since the age of 18 years, change in weight since the age of 35 years, history of fracture after the age of 55 years, race/ethnicity, physical activity, smoking, history of parental fracture after the age of 40 years, diabetes treated with medications, and corticosteroid use
[31] Age, sex and race/study center, body mass index, sports-activity tertile, alcohol consumption, cigarette smoking, and medication use.
[32] Plasma PTH serum 25(OH)D3 concentration, concomitant diseases (hypertension, ischemic heart disease and diabetes mellitus), smoking status, age, gender and season.
[33] Age-sex-race adjusted
[34] Gender, race-ethnicity, age, calendar year, urban/rural, geographic location, median income, previous fracture, other predisposing conditions (glucocorticoid related, fall-related, renal disease, depressive illness, AMI, other heart disease, bone disease, cancer)
[35] Matched by age, sex, and GP practice.

Table 3.3 Author's Judgement rating on Quality Assessment for cohort studies using Newcastle-Ottawa tool

Study, year	Review	S 1	S 2	S 3	S 4	Comp	Out 1	Out 2	Out 3	Tot
Ahmed, 2006	hip and NV	*	*	*	*	**	*	*	-	8
Berry, 2017	hip	-	*	*	*	**	*	*	-	7
Bonds, 2006	NV	-	*	*	*	**	-	*	-	6
Chen, 2008	hip	*	*	*	*	**	*	*	*	9
de Liefde, 2005	hip and NV	*	*	*	*	**	*	*	-	8
Dobnig, 2006	hip	-	*	*	*	**	*	*	-	7
Forsen,1999	hip	*	*	*	*	**	*	*	-	8
Hamilton, 2017a	hip	-	*	-	*	**	*	*	-	6
Hamilton, 2017b	hip	-	*	-	*	**	*	*	-	6
Hippisley-Cox, 2012	hip	*	*	*	*	**	*	*	*	9
Holm, 2018	hip and NV	*	*	*	*	**	-	*	*	8
Holmberg, 2006	hip and NV	*	*	*	*	**	*	*	*	9
Hothersall, 2014	hip	*	*	*	*	**	*	*	-	8
Ivers, 2001	hip and NV	-	*	*	*	**	*	*	-	7
Janghorbani 2006	hip	-	*	-	*	**	-	*	*	6
Jorgensen, 2014	hip	*	*	*	*	**	*	*	*	9
Jung, 2012	NV	-	*	*	*	**	*	*	-	7
Kim, 2017	hip and NV	*	*	*	*	**	*	*	*	9
Koh, 2010	hip	*	*	*	*	**	*	*	*	9
Lai, 2015	hip	*	*	*	*	**	*	*	*	9
Lee, 2015 (EPESE)	hip and NV	*	*	*	*	**	-	*	-	7
Lee, 2018	hip	-	*	*	*	**	*	*	-	7
Leslie, 2007	hip	*	*	*	*	**	*	*	*	9
Leslie, 2014	hip	*	*	*	*	**	*	*	*	9
Li, 2019	hip	-	*	*	*	**	*	*	-	7
Lipscombe, 2007	hip	*	*	*	*	**	*	*	-	8
Looker, 2016	hip	*	*	*	*	**	*	*	*	9

Study, year	Review	S 1	S 2	S 3	S 4	Comp	Out 1	Out 2	Out 3	Tot
Majumdar, 2016	hip	*	*	*	*	**	*	*	*	9
Martinez-Laguna, 2015	hip	*	*	*	*	**	*	*	*	9
Meyer, 1993	hip	*	*	*	*	**	*	*	*	9
Napoli, 2014	NV	-	*	*	*	**	*	*	-	7
Nicodemus, 2001	hip	-	*	-	*	**	-	*	-	5
Oei, 2013	hip and NV	*	*	*	*	**	*	*	-	8
Ottenbacher 2002	hip	*	*	*	*	**	-	*	-	7
Rathmann, 2015	hip and NV	*	*	*	*	**	*	*	-	8
Reyes, 2014	hip	*	*	*	*	**	*	*	*	9
Robbins, 2007	hip	-	*	-	*	**	*	*	-	6
Schafer, 2010	NV	-	*	*	*	**	*	*	-	7
Schneider, 2013	hip and NV	-	*	*	*	**	*	*	-	7
Schwartz 2001	hip and NV	-	*	*	*	**	*	*	-	7
Strotmeyer, 2011	hip	-	*	*	*	**	*	*	-	7
Taylor, 2011	hip and NV	*	*	*	*	**	*	*	-	8
Wallander, 2017	hip and NV	*	*	*	*	**	*	*	*	9
Weber, 2015	hip	*	*	*	*	**	*	*	-	8

S selection; Comp comparability; Out outcome; NV non-vertebral; Tot total

Justification for each criterion

Selection 1: representativeness of exposed cohort (representative of person with DM at risk of fractures) (where exposure is MD)

*a) truly representative of the average person with DM in the community *

*b) somewhat representative of the average person with DM in the community * (e.g. if some patient groups excluded, or used a database which only included a subset of the population, such as those with health insurance)

c) selected group of users eg nurses, volunteers (where that selection results in patients likely to have different outcomes to whole population)

d) no description of the derivation of the cohort

Selection 2: Selection of the non-exposed cohort

*a) drawn from the same community as the exposed cohort *

b) drawn from a different source

c) no description of the derivation of the non-exposed cohort

Selection 3: Ascertainment of exposure (i.e. DM)

*a) secure record (eg surgical records) *

*b) structured interview *

c) written self-report

d) no description

Selection 4: Demonstration outcome of interest not present at study start

*a) yes *

b) no

Comparability: (one star if study controls for age, two stars if study controls for gender)

Study can score up to two stars

*a) study controls for age (select the most important factor) *

b) study controls for gender

Outcome 1: Assessment of outcome

*a) independent blind assessment *

*b) record linkage * (e.g. hospital records)

c) self-report

d) no description

Outcome 2: Length of follow-up (1 year minimum)

*a) yes (select an adequate follow up period for outcome of interest) *

b) no

Outcome 3: Adequacy of follow-up

*a) complete follow up - all subjects accounted for *

*b) subjects lost to follow up unlikely to introduce bias - small number lost - ≥80% follow up, or description provided of those lost) *

c) follow up rate <80% (select an adequate %) and no description of those lost

d) no statement

Table 3.4 Author's Judgement rating on Quality Assessment for case-control studies using Newcastle-Ottawa tool

Study, year	Review	Sel 1	Sel 2	Sel 3	Sel 4	Comp	Exp 1	Exp 2	Exp 3	Total
Gerber 2013	hip	*	*	*	-	**	*	*	*	8
Poor, 1995	hip	*	*	*	-	**	*	*	*	8
Segal, 2009	hip	*	*	*	-	**	-	*	-	6
Keegan 2002	NV	*	*	*	*	**	*	*	*	9

Sel selection; Comp comparability; Exp exposure; NV non-vertebral;

Justification for each criterion

Selection 1: Is case definition adequate? (i.e. ascertainment of fracture)

a) Requires some independent validation (e.g. >1 person/record/time/process to extract information, or reference to primary record source such as x-rays or medical/hospital records)

b) Record linkage (e.g. ICD codes in database) or self-report with no reference to primary record

c) No description

Selection 2: Representativeness of cases

a) All (i.e. consecutive) eligible cases with outcome of interest over a defined period of time, all cases in a defined catchment area, all cases in a defined hospital or clinic, group of hospitals, health maintenance organisation, or an appropriate sample of those cases (e.g. random sample)

b) Not satisfying requirements in part (a), or not stated.

Selection 3: Selection of controls

This item assesses whether the control series used in the study is derived from the same population as the cases and essentially would have been cases had the outcome been present.

a) Community controls (i.e. same community as cases and would be cases if had outcome)

b) Hospital controls, within same community as cases (i.e. not another city) but derived from a hospitalised population

c) No description

Selection 4: Definition of controls

a) If cases are first occurrence of outcome, then it must explicitly state that controls have no history of this outcome. If cases have new (not necessarily first) occurrence of outcome, then controls with previous occurrences of outcome of interest should not be excluded.

b) No mention of history of outcome

Comparability 1 (one star if study controls for age, two stars if study controls for gender)

A maximum of 2 stars can be allotted in this category. Either cases and controls must be matched in the design and/or confounders must be adjusted for in the analysis. Statements of no differences between groups or that differences were not statistically significant are not sufficient for establishing comparability. Note: If the odds ratio for the exposure of interest is adjusted for the confounders listed, then the groups will be considered to be comparable on each variable used in the adjustment.

* Age

*Gender

Exposure 1: Ascertainment of exposure (i.e. Diabetes)

*a) secure record (eg surgical records) *

*b) structured interview where blind to case/control status *

c) interview not blinded to case/control status

d) written self-report or medical record only

e) no description

Exposure 2: Same method of ascertainment for cases and controls

*a) yes *

b) no

Exposure 3: Non-response rate

*a) same rate for both groups *

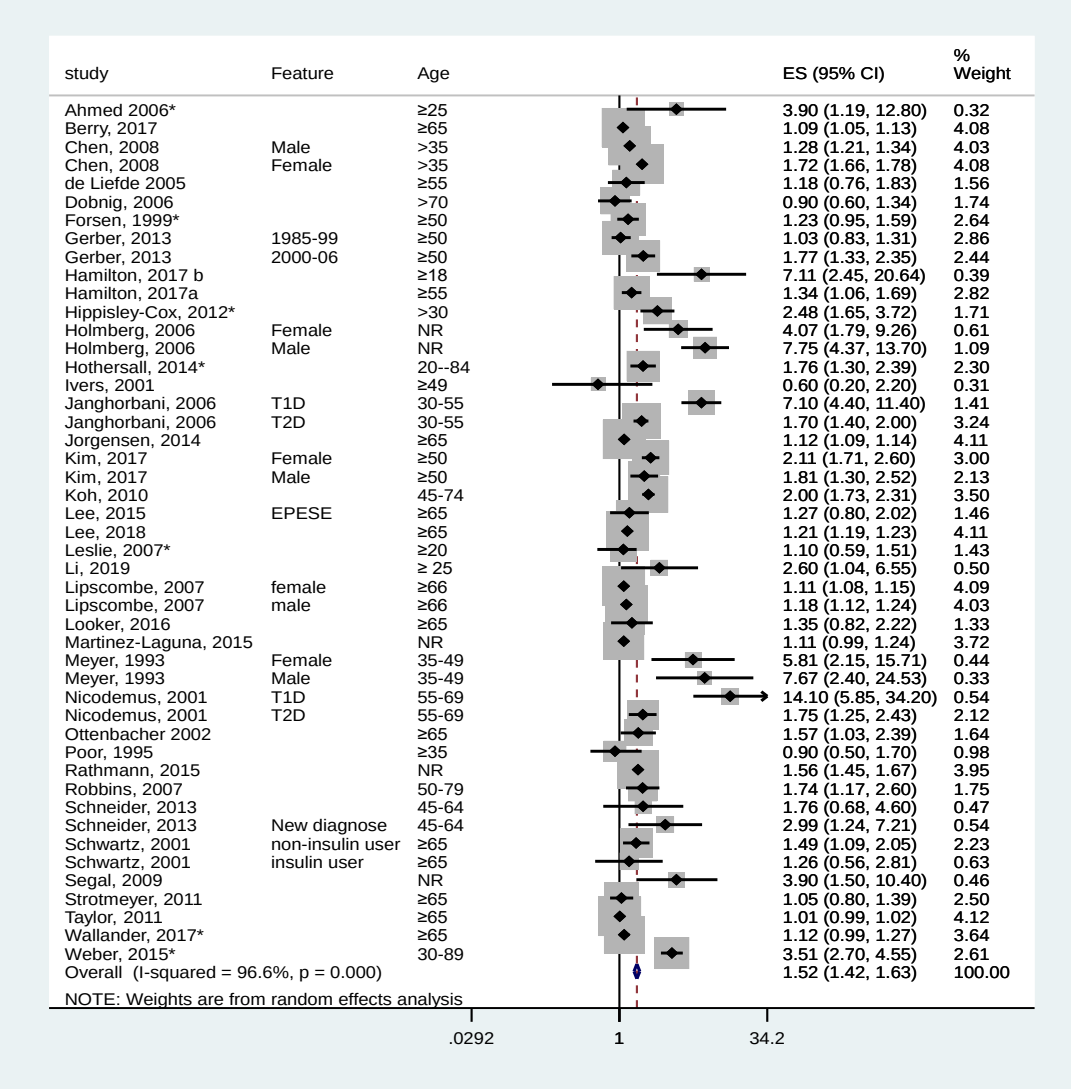
b) non-respondents described

c) rate different and no designation

Meta-analysis results

Hip fractures

The overall RR of hip fractures was increased in diabetes. Forty-three studies were included in the hip fractures analyses. These meta-analyses report the data of 17,575,873 participants, 2,387,899 with DM and 321,720 fractures. As overlapping population were avoided, 37 studies were included in the overall analysis, resulting in a RR of 1.52, 95%CI 1.42-1.63 and high heterogeneity (I^2 96.6% $p<0.001$) (Figure 3-2). We explored the heterogeneity using subgroup and sensitivity analyses and meta-regression.

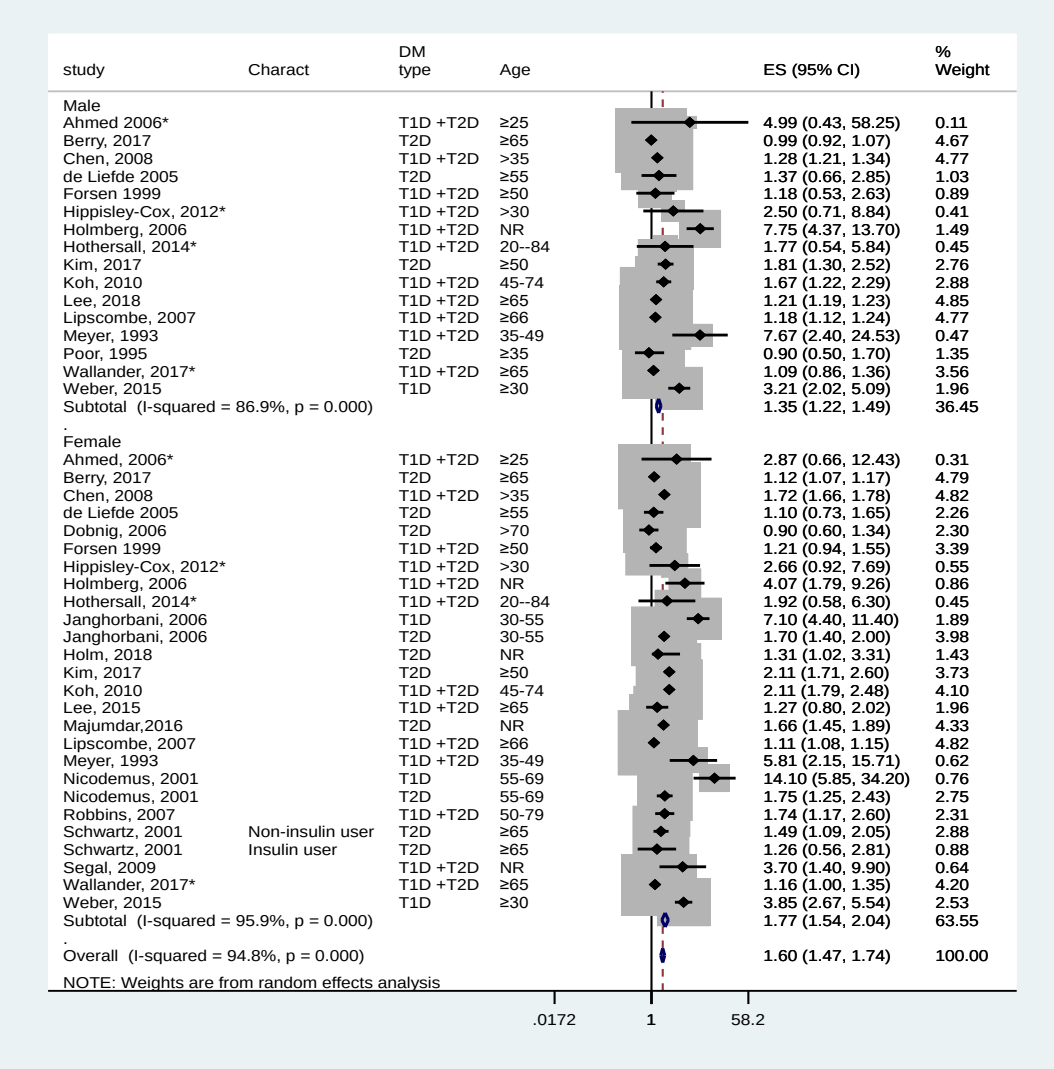


* Summarised using random-effects model

Figure 3-2 Forest plot overall hip fractures risk in diabetes

Subgroup analysis by gender

The RR was significantly higher in females (RR 1.77, 95%CI 1.54-2.04) than in males (RR 1.35, 95%CI 1.22-1.49). This analysis pooled data from ten single-gender studies (64, 91, 92, 107, 113, 119, 122, 129, 131, 133) and the studies that reported gender-specific risk (15, 90, 93, 103-105, 108, 112, 115, 117, 118, 124, 128, 138). One study reported an overall risk estimate without a gender-specific risk and the risk in females and the latter was used in this analysis (135). The studies that did not report a gender-specific risk were not included. In total, 25 studies were summarised. Heterogeneity remained high (I^2 94.8% p<0.001) (Figure 3-3).



* Summarised using random-effects model

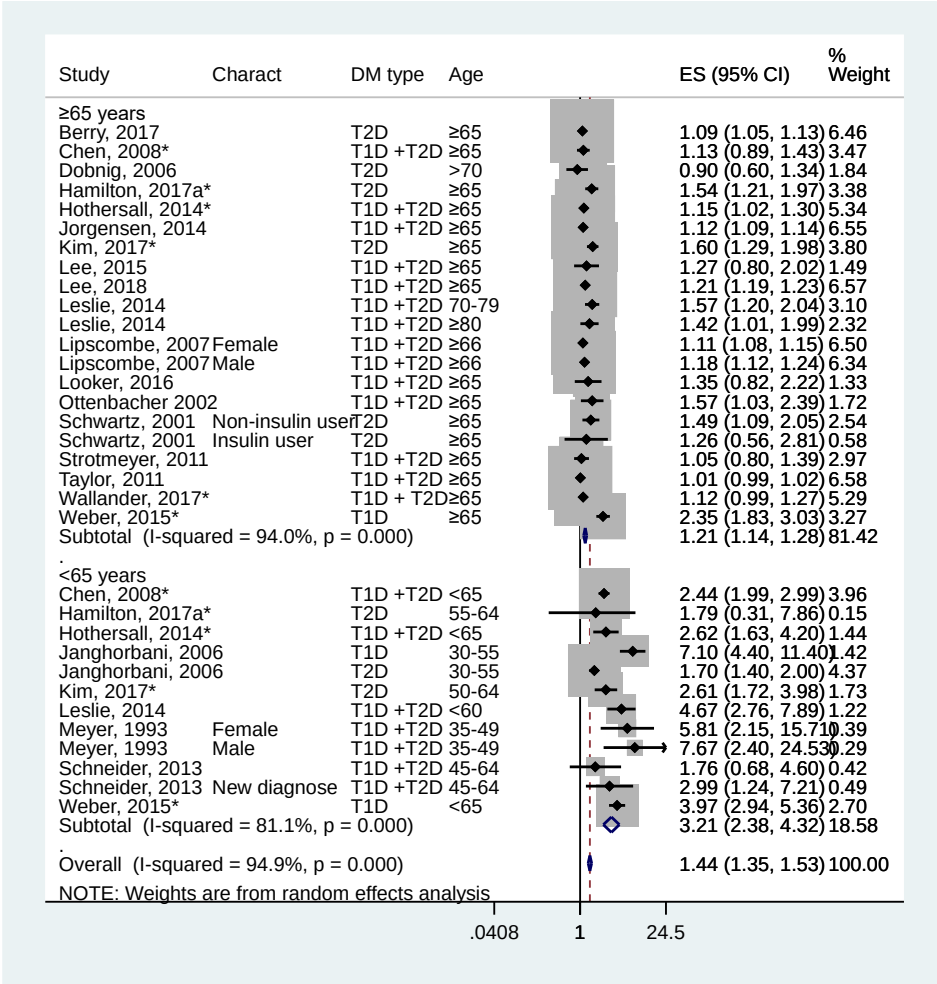
Figure 3-3 Forest plot hip fractures risk in Diabetes by gender

Subgroup analysis by age

The RR was higher in younger populations. The included studies reported several age-ranges and they were grouped in two ways; people younger and older that 65 years-old and in narrower age ranges, namely < 50 years, 50-59 years, 60-69 years, 70-79 years, and older than 80 years.

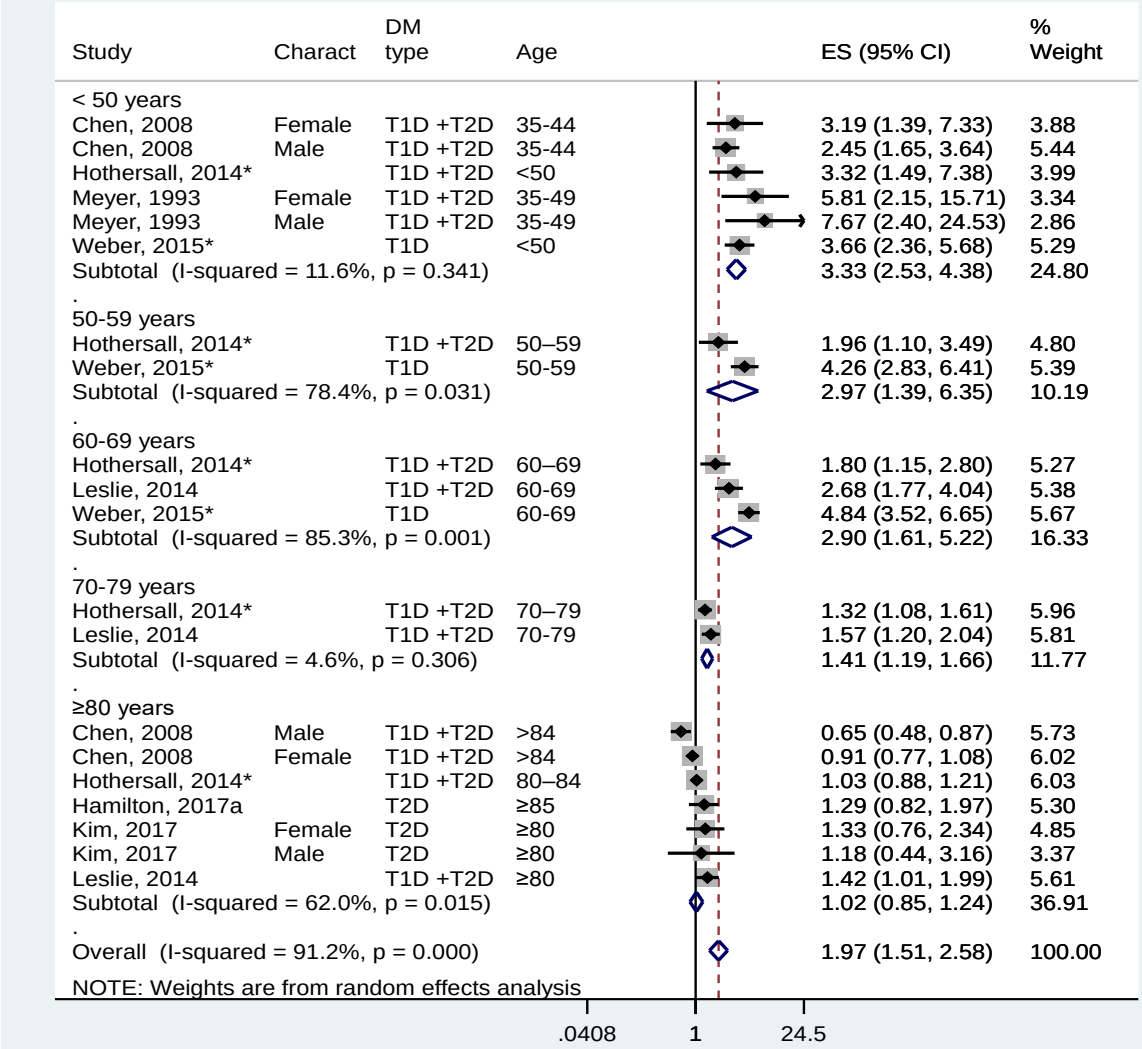
When using the cut-off of 65 years old, the RR was significantly higher in the younger population; RR 3.21, 95%CI 2.38-4.32 in participants younger than 65 years-old and RR 1.21, 95%CI 1.14-1.28 in participants older than 65 years-old with high heterogeneity (I^2 96.6% p<0.001) (Figure 3-4). Interestingly, the RR seems to decrease progressively with ageing. Seven studies reported the risk by similar age ranges and they were clustered in narrower age

ranges. The relative risk decreased progressively from RR 3.33, 95%CI 2.53-4.38 in the population < 50 years, to RR 2.97, 95%CI 1.39-6.35 in 50-59 years; RR 2.90, 95%CI 1.61-5.22 in 60-69 years; RR 1.41, 95%CI 1.19-1.66 in 70-79 years and reached no increase in the risk in the population older than 80 years RR 1.02, 95%CI 0.85-1.24. Heterogeneity was high (I^2 91.2, p<0.001). The analysis using the RRR showed that, despite overlapping confidence intervals, the RR was significantly lower in the 70-79 years group compared to 60-69 years group, and in the ≥ 80 years group compared to 70-79 years (Figure 3-5).



* Summarised using random-effects model

Figure 3-4 Forest plot hip fractures risk in Diabetes by age (< 65 years vs >65years)

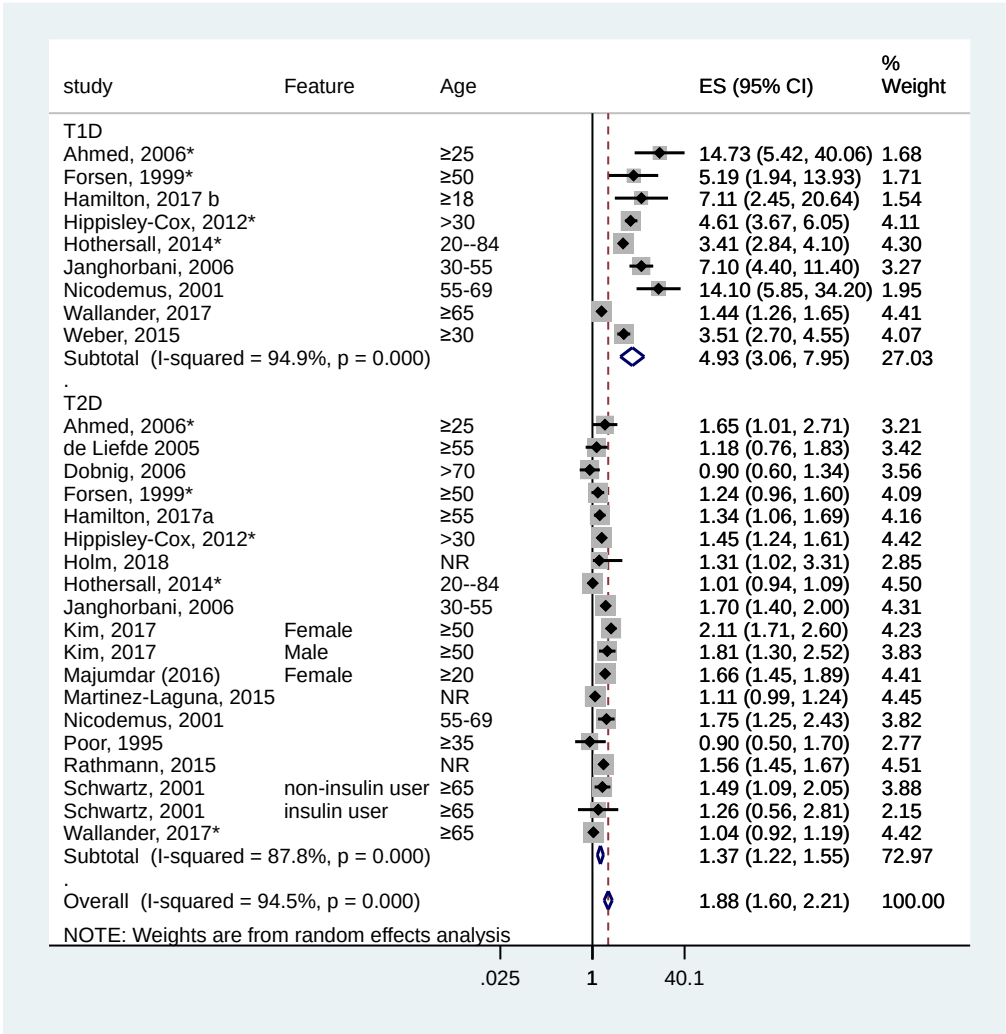


* Summarised using random-effects model

Figure 3-5 Forest plot hip fractures risk in Diabetes by age-range

Subgroup analysis by diabetes type

The RR was significantly higher in T1D (RR 4.93, 95% CI 3.06-7.95) than in T2D (RR 1.37, 95% CI 1.22-2.21). This analysis summarised the data from the 19 studies that reported the risk for specific diabetes type. Overall heterogeneity was high (I^2 94.5% and p< 0.001) (Figure 3-6).

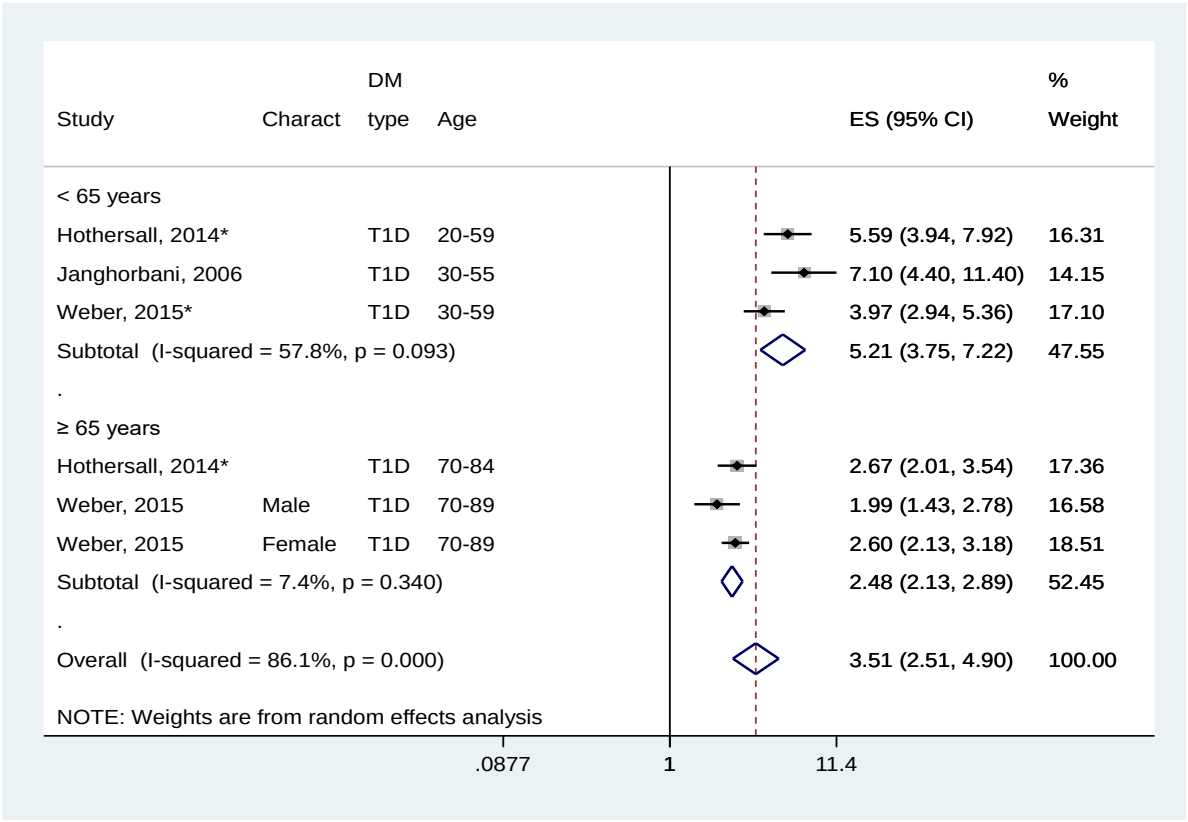


* Summarised using random-effects model

Figure 3-6 Forest plot hip fractures risk in Diabetes by type

Additional exploratory analysis by age and DM type

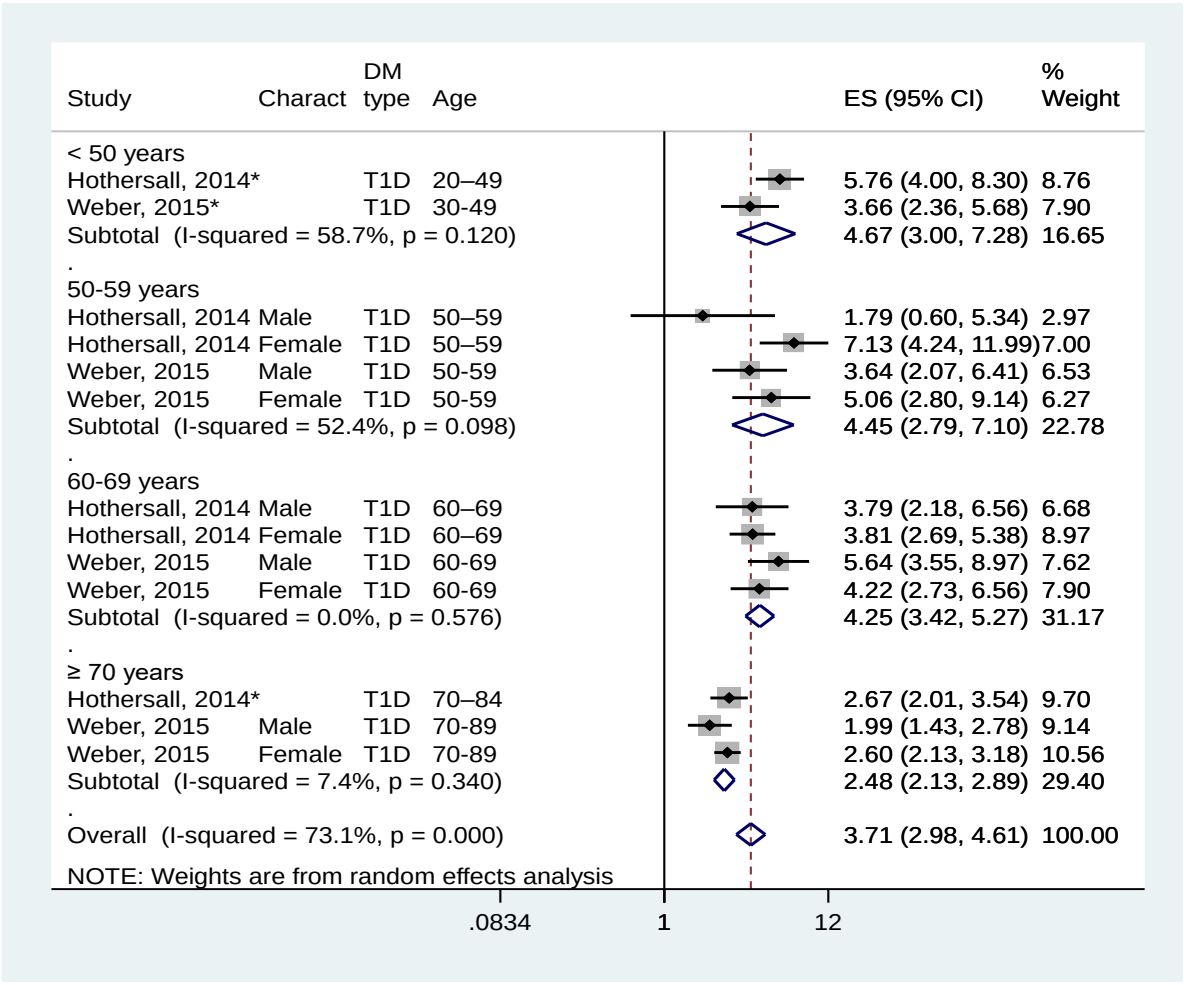
As age and diabetes type seemed to have an important impact on the risk of fractures in diabetes, we ran the age subgroup analysis for each diabetes type. In the T1D group, the risk of hip fracture was higher in the younger than 65 years old (RR 5.21, 95%CI 3.75-7.22) than in the older than 65 years old (RR 2.48, 95%CI 2.13-2.89) Figure 3-7.



* Summarised using random-effects model

Figure 3-7 Forest plot hip fractures risk in T1D by type age (65 years vs > 65 years)

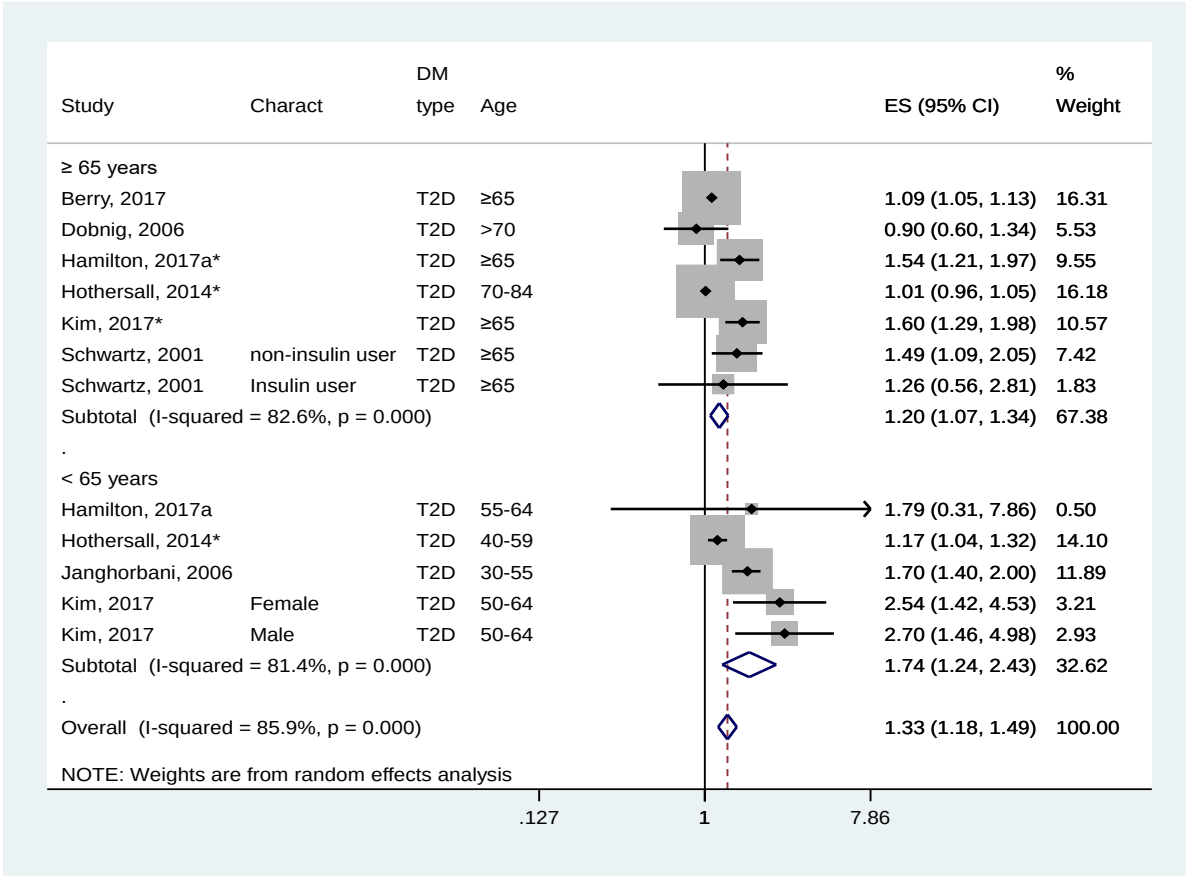
The analysis for age ranges showed similar results. The risk of hip fractures was similar in the younger than 50 years old (RR 4.67, 95%CI 3.00-7.28), in the individuals between 50-59 years old (RR 4.45, 95%CI 2.79-7.10) and in the ones at 60-69 years old (RR 4.25, 95%CI 3.42-5.27), but significantly lower in the individuals older than 70 years old (RR 2.48, 95%CI 2.13-2.89). To allow the inclusion of more studies, we used > 70 years as the older group (Figure 3-8).



* Summarised using random-effects model

Figure 3-8 Forest plot hip fractures risk in T1D by age range

In T2D, the risk of hip fractures was also higher in the younger (RR 1.74, 95% CI 1.24-2.43) than the older than 65 years old, (RR 1.20, 95% CI 1.07-1.34); (RRR 0.68 95%CI 0.48-0.98) (Figure 3-9). There were not enough studies with data by age range exclusively in T2D to perform the T2D analysis by age range.



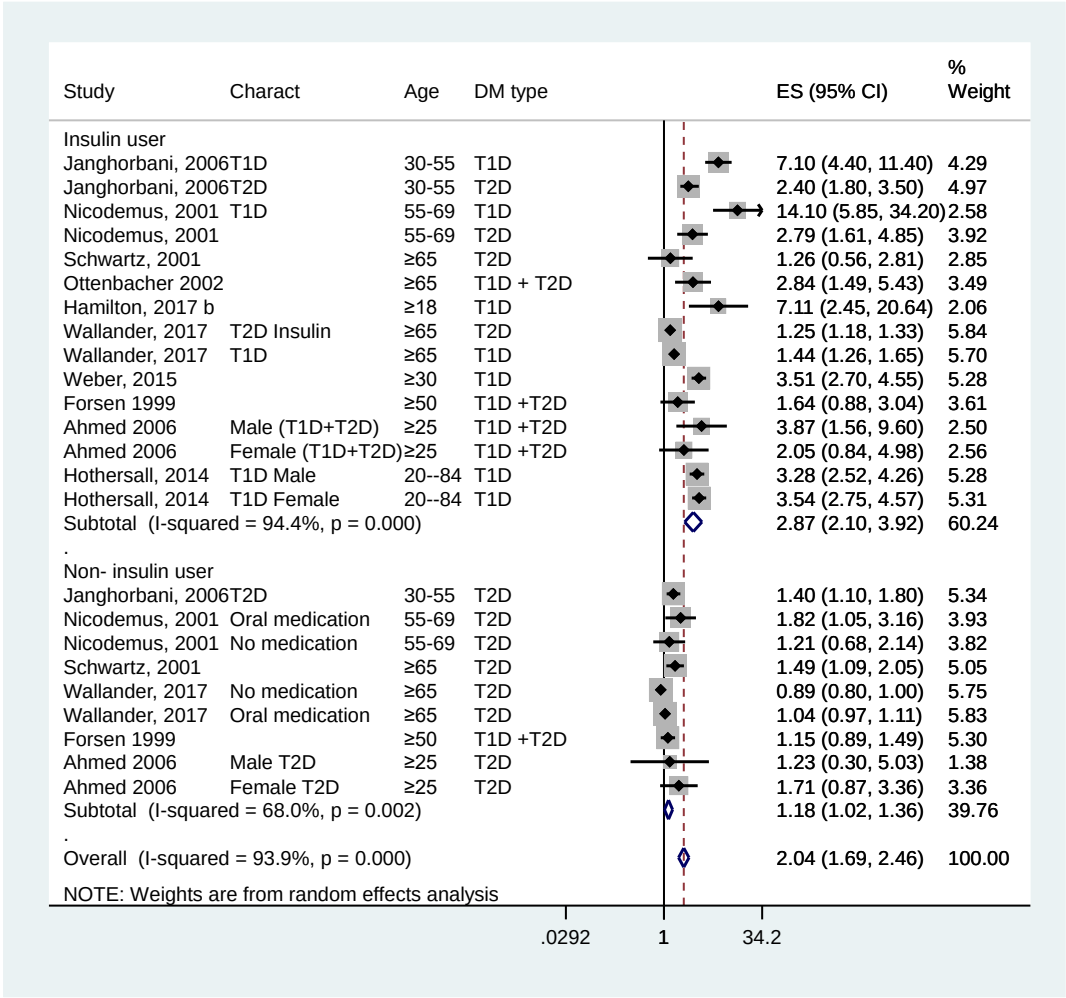
* Summarised using random-effects model

Figure 3-9 Forest plot hip fractures risk in T2D by age (65 years vs > 65 years)

Subgroup analysis by insulin use

The insulin users had a higher RR of hip fractures (RR 2.87, 95%CI 2.10-3.92) than non-insulin users (RR 1.18, 95%CI 1.02-1.36). Ten studies were summarised in this analysis. Insulin users included T1D and T2D insulin users and non-insulin users included just T2D. Heterogeneity was high (I^2 93.9%, p<0.001) (Figure 3-10).

This analysis included patients with both T1D and T2D. Although both of them use insulin this is a heterogeneous group. T1D patients do not produce insulin and need exogenous insulin for treatment from the onset of the disease. Conversely, T2D patients have insulin resistance and insulin treatment is added when other treatment options fail. Therefore, these are patients with advanced T2D. Despite the differences, both groups are affected by hypoglycaemia that increases the risk of falls and fractures. Therefore, the analysis by insulin use probably does not reflect the effects of insulin in bone, but its indications and adverse effects.



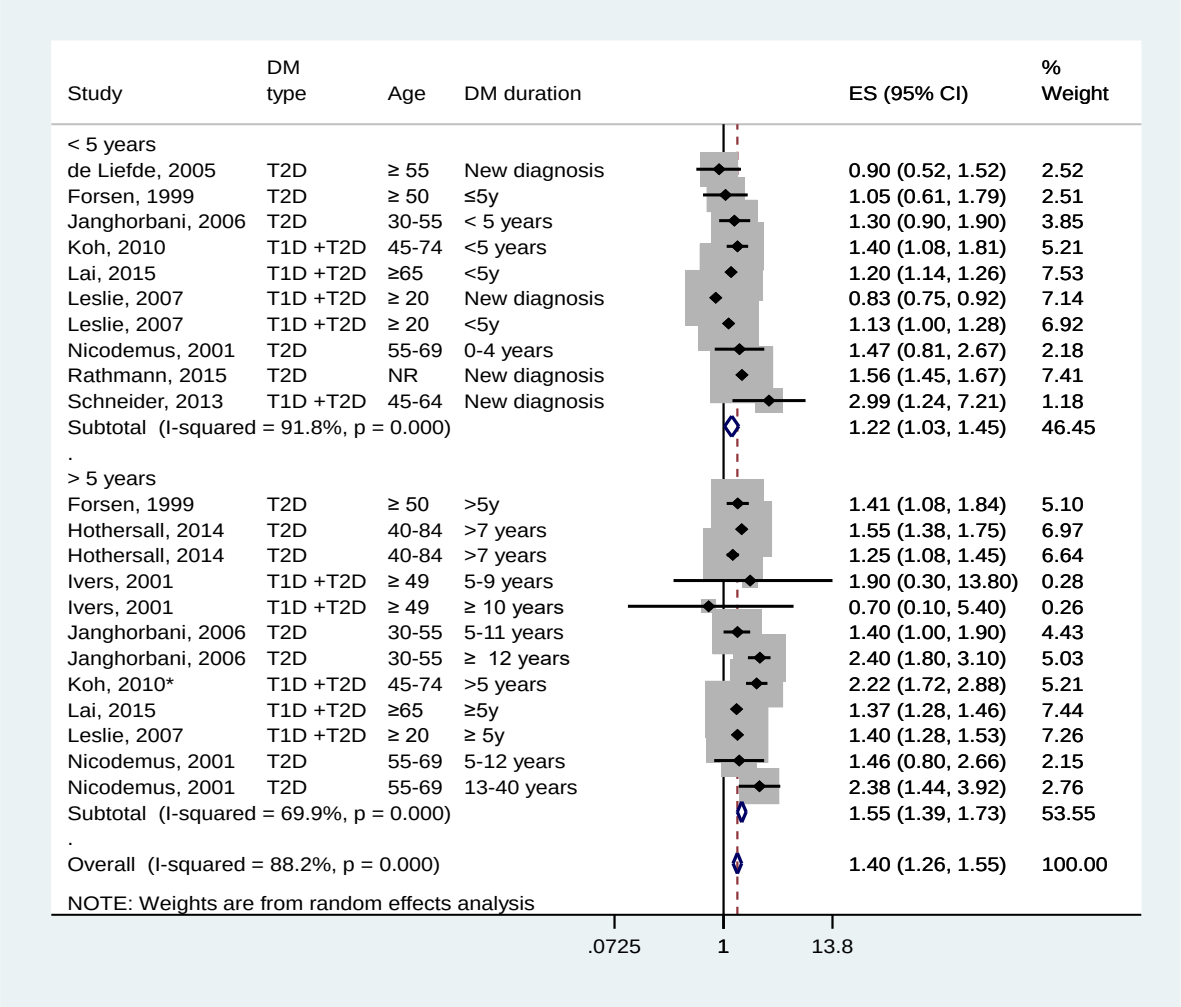
* Summarised using random-effects model

Figure 3-10 Forest plot hip fractures risk in Diabetes by insulin use

Subgroup analysis by diabetes duration

The risk of fractures increased progressively with the increase in diabetes duration. Due to availability of data, the studies were grouped using 5- and 10-years duration thresholds.

Data from people with diabetes for less than five years and more than five years were grouped, including 11 studies. People with diabetes for less than five years had a lower RR of hip fractures (RR 1.22 95%CI 1.03-1.45) than people with diabetes for more than five years (RR 1.55 95%CI 1.39-1.73), (RRR 0.79 95%CI 0.64-0.96) (Figure 3-11). Heterogeneity was high (I^2 88.2% p<0.001).



* Summarised using random-effects model

Figure 3-11 Forest plot hip fractures risk in Diabetes by diabetes duration (5 years cut-off)

Additionally, a 10-years duration cut-off was used. Ten studies were included and a shorter duration (< 10 years) was associated with a lower RR of hip fractures RR 1.30, 95%CI 1.10-1.54 than a longer duration (> 10 years) RR 2.42, 95%CI 2.08-2.81. High heterogeneity was observed I^2 92.2% p<0.001 (Figure 3-12).

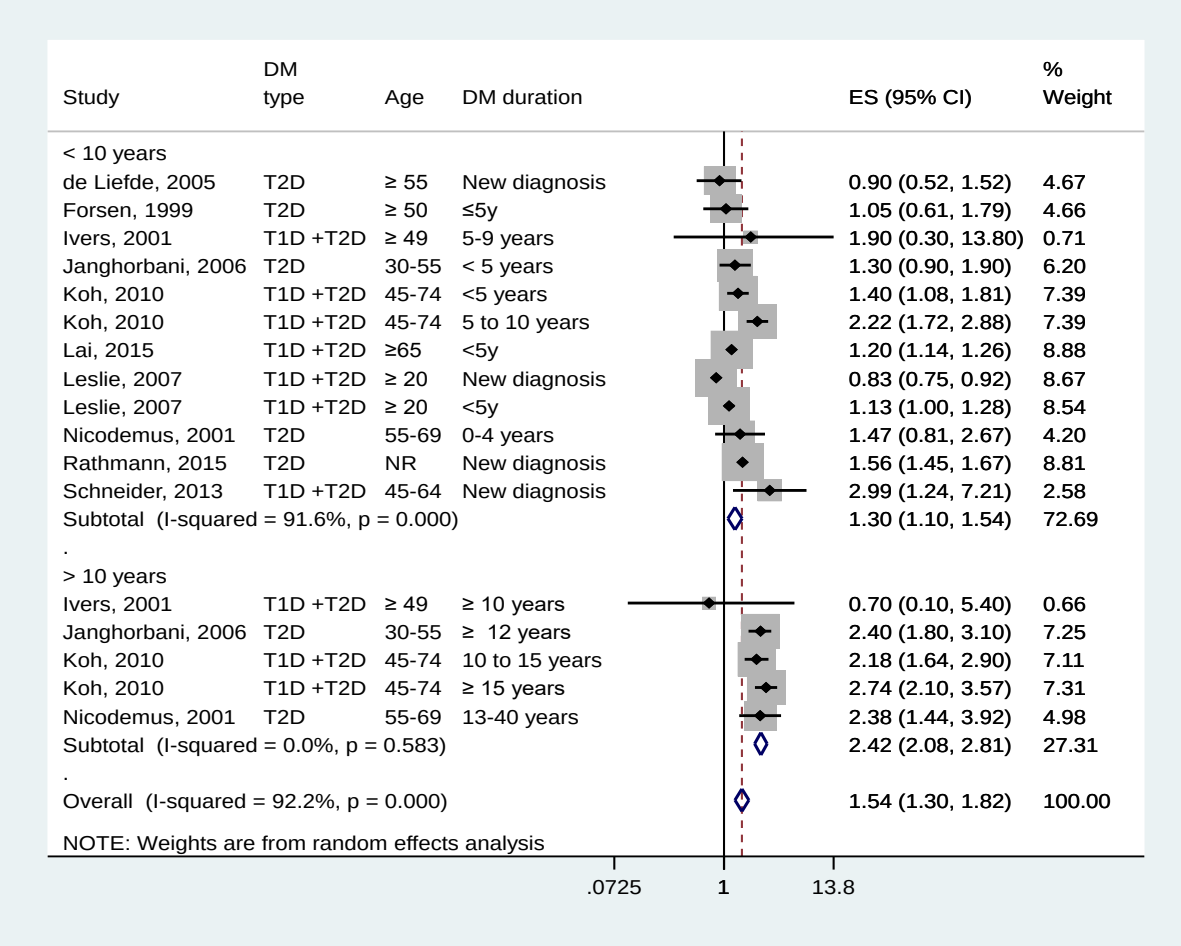


Figure 3-12 Forest plot hip fractures risk in Diabetes by diabetes duration (10 years cut-off)

We were able to show an increase in the risk of fractures with increased diabetes duration. However, this analysis has limitations. The number of studies that reported the risk of fractures according to diabetes duration was limited. In addition, studies reported the duration using different metrics (e.g. new diagnosis and previous diagnosis, 5 yeas, 5-11 years, …) what made summarising the data a challenge. Finally, in T2D the diagnosis might be missed for years, so the data might not reflect the real disease duration.

Subgroup analysis by BMI

Four studies reported the risk of fractures by BMI and we assessed the risk by BMI range. There was no significant difference between the groups, but the forest plot showed a trend to a decrease in the RR of hip fractures with the increase in BMI, namely in low/ normal weight (BMI< 25kg/m^2) the RR was 1.69 95%CI 1.08-2.63; in overweight people (BMI 25-30 kg/m^2) the RR was 1.18 95%CI 0.98-1.42, and in obese people (BMI > 30 kg/m^2) RR was 0.96 95%CI 0.58-1.59. The number of studies was limited and there was a considerable overlap in the confidence intervals (Figure 3-13).

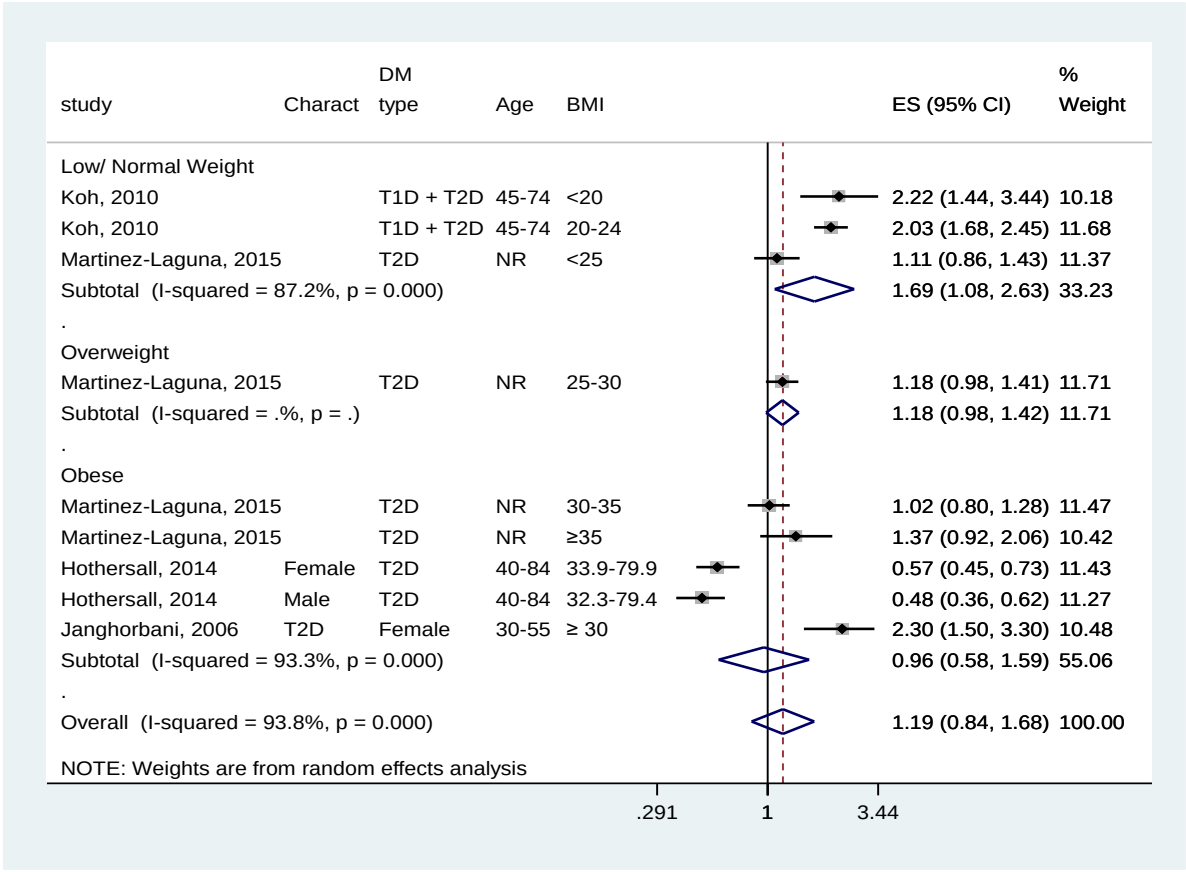
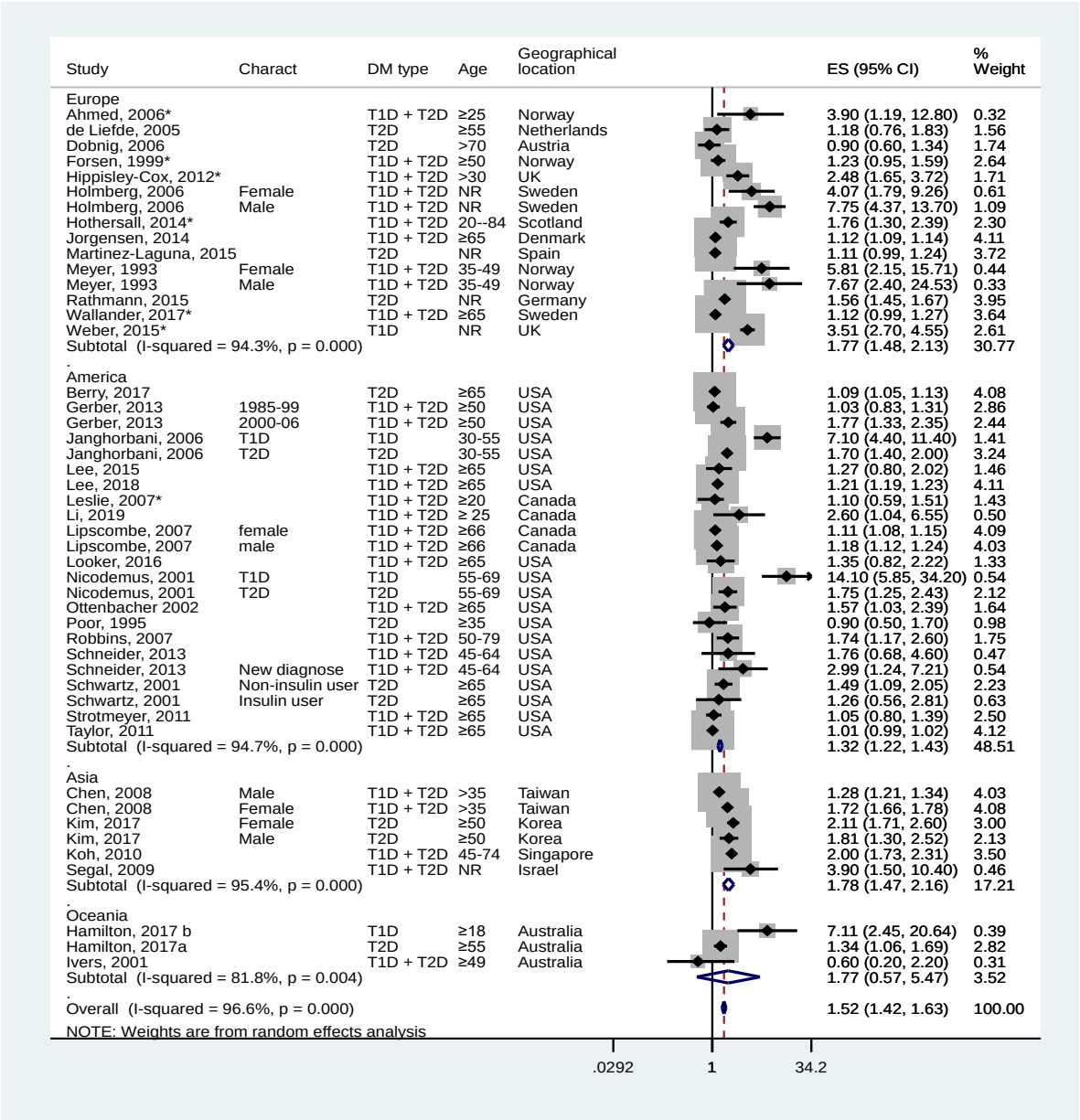


Figure 3-13 Forest plot hip fractures risk in Diabetes by BMI range

Subgroup analysis by continent

The studies were grouped according to the geographical location. The risk was similar in Europe (RR 1.77 95%CI 1.48-2.13) and Asia (RR 1.78 95%CI 1.47-2.16) but lower in America (RR 1.32 95%CI 1.22- 1.43). The risk estimates from Oceania, all Australian studies, produced a wide confidence interval (RR 1.77 95%CI 0.57-5.47). The heterogeneity was high (I^2 96.6% and p<0.001) (Figure 3-14).



* Summarised using random-effects model

Figure 3-14 Forest plot hip fractures risk in Diabetes by geographical location

Subgroup analyses not performed

Few studies addressed the effect of diabetes control or microvascular complications on the risk of fractures, therefore, it was not possible to perform subgroup analysis. Oei et al reported data on the risk of hip, wrist and all types of fractures in diabetes by metabolic control. Participants with inadequate control had an increase in the risk of fractures compared to participants with adequate diabetic control or participants without diabetes (144). This pattern was observed in all fractures and wrist fractures, but the analysis for hip fracture subtype was inconsistent. However, the number of hip fractures in the participants with diabetes with adequate (n=15) and inadequate (n=11) control was small, suggesting that the study probably did not have the power to assess this risk.

For microvascular complications, Lee et al investigated the effect of neuropathy in the risk of hip and any fractures. The authors developed a model that evaluated comorbidities as potential mediating factors for fractures. In this model, neuropathy explained around 20% of the risk of hip and any fractures, however, the analysis was not restricted to diabetic neuropathy (64).

Sensitivity analysis

We ran the analyses excluding one study at a time and no important variation was observed in the results. The RR varied from RR 1.48, 95%CI 1.38-1.58 to RR 1.57, 95%CI 1.46-1.70 and heterogeneity remained high suggesting that no study had an important individual impact in the results. We excluded the case-control studies, and the RR and heterogeneity also remained very similar (RR1.53, 95%CI 1.42-1.65). Sensitivity analysis excluding studies from Australia showed similar RR (RR 1.52, 95%CI 1.41-1.63). Finally, a sensitivity analysis excluding each kind of risk estimate (i.e. OR, HR) also resulted in similar results with the RR varying from RR 1.40 95%CI 1.30-1.50 to RR 1.58 95%CI 1.45-1.72.

Meta-regression

Meta-regression analysis suggested that diabetes type and age contribute substantially to the RR. In the DM type subgroup analysis, diabetes type accounted for 60% of the RR. In the age range analysis (65-years cut-off), age accounted for 48% of the RR. In the analysis combining both age and DM type, they accounted for 83% of the RR of hip fractures in diabetes.

Funnel plots

Visual analysis of the funnel plot was not suggestive of publication bias, despite asymmetry (Figure 3-15). The plots are scattered through both the significant and non-significant results areas suggesting that even studies with non-significant results were published and captured by our research. The plots are also scattered from the apex to the bottom of the triangle, which reflect the size of the studies and shows that big (near the apex) and small (towards the bottom) studies with significant and non-significant results were included. Symmetrical inverted funnels are expected in data from interventional studies, in the absence of bias and between study heterogeneity as the scatter will be due to the sample variation (101). Heterogeneity might add horizontal scatter to the funnel plot and if heterogeneity is large it might overwhelm the sampling error and make the plot appears cylindrical (101). The high heterogeneity found in the analyses might have affected the funnel plots shape.

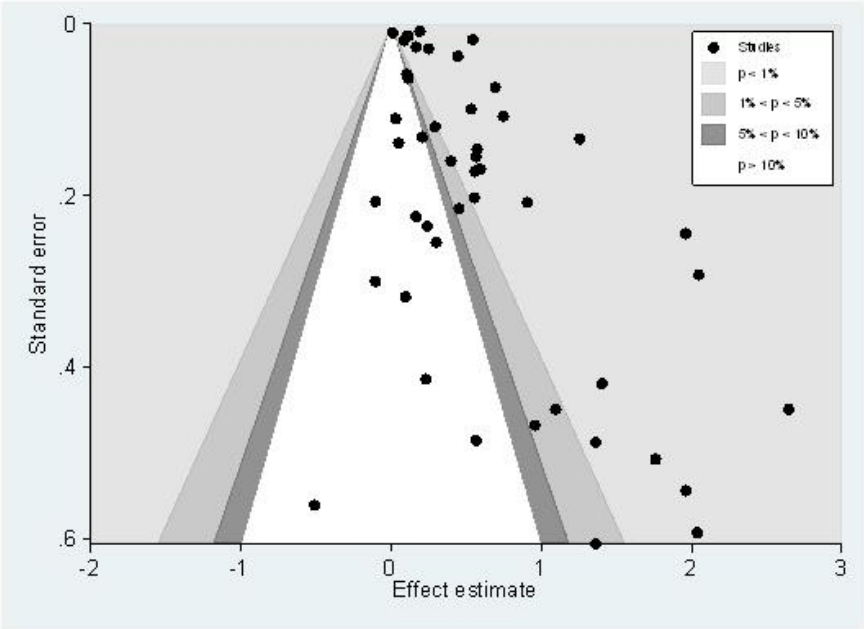


Figure 3-15 Funnel plot studies included in the hip fractures analyses

Non-vertebral fractures

Non-vertebral fracture study characteristics

Table 3.5 summarises the study characteristics. Eighteen studies reported the risk of fractures in two or more sites and were included in the non-vertebral fractures risk analysis (90, 92, 93, 103, 113, 115-117, 119, 132, 134, 137, 139-144). All but one study (141) were cohorts, 12

prospective (90, 92, 93, 103, 115, 116, 119, 134, 139, 142-144) and five retrospective (113, 117, 132, 137, 140). Eight studies were from the USA (92, 119, 134, 137, 139, 141-143), seven from Europe (one from Norway (103); two from the Netherlands (90, 144); one from Denmark (113); two from Sweden (93, 115) and one from Germany (132)); two Korean studies from Asia (117, 140) and one study from Australia (116). Nine studies did not specify diabetes type (115, 116, 119, 134, 137, 140-143), while seven reported data just from T2D (90, 92, 113, 117, 132, 139, 144) and two from both types (93, 103). There was no study reporting data just from T1D participants. Five studies reported data just from women (92, 113, 119, 139, 140), one just from men (142) and the others from both genders (90, 93, 103, 115-117, 132, 134, 137, 141, 143, 144). The age range varied from 20 to 98 years-old and the follow-up from a median of 1.3 years to 20 years. The study size varied from 1,949 (143) to 1,694,051 participants (137). Although other ethnicities were included, such as Asian, blacks, Hispanics and others (117, 119, 134, 139, 140, 142, 143, 146), the majority of the data addressed white populations. Nine studies reported the risk of non-vertebral fractures as a category (90, 92, 93, 103, 116, 117, 140, 142, 143) and the others reported several combinations of sites including axial and peripheral sites. Only one study did not include the hip site in its pool (141).

Non-vertebral fracture quality assessment

We report a summary of the authors' judgement in tables Table 3.3 (cohort) and Table 3.4 (case-control). As in the hip fractures analysis, overall the quality of the studies was good and most of the studies scored higher than seven. Once more, the main criteria not addressed by the studies were the adequacy of follow-up (90, 92, 103, 116, 119, 132, 134, 137, 139, 140, 142-144), not reported in most of the studies; and representativeness of the cohort (92, 116, 134, 139, 140, 142, 143), mainly due to the recruitment of volunteers. One cohort study scored six and lost scores in representativeness, assessment of the outcome and adequacy of the follow-up. This study selected volunteers, fractures were self-reported and the follow-up was not reported (139).

Table 3.5 Non-vertebral fractures studies characteristics

Study, year	Study design	Country	Cohort name	DM type	Age (y)	Fol up y (SD)	Pop total/ DM	Fract	Ethnicity	F (%)	fracture sites included	Risk estimate's group	Risk estimate
Ahmed, 2006	Cohort[1]	Norway	The Tromsø study	Both	25-98	6	27,159/ 455	1,249	NR	52	Non-vertebral	Calculated overall	1.56 (0.84-2.90)[3]
Bonds, 2006	Cohort[1]	USA	WHI-OS	T2D	50-79	7	93,405/ 5285	NR	NHW 83.2% Black 8.1% Hispanic 3.8% American Indian 0.4% Asian/Pacific Islander 3.1% Unknown 1.4%	100	Hip/pelvis/upper leg, Lower leg/ankle/knee, Foot, Upper arm/shoulder/ elbow, Lower arm/wrist/ hand	Calculated overall	1.28 (1.11-1.47)[4]
de Liefde, 2005	Cohort[1]	Nether-lands	The Rotterdam Study	T2D	≥55	6.8 (2.3)	6,655/ 792	771	NR	60	Non-vertebral	Overall	1.18 (0.92–1.52)[5]
Oei, 2013	Cohort[1]	Nether-lands	The Rotterdam Study	T2D	≥55	12 (4.2)	4,135/ 420	1,068	NR	60	Hip, wrist	Calculated overall	1.12 (0.83-1.53)[6]
Holm, 2018	Cohort[2]	Denmark	Danish National registries	T2D	NR	5.8 (NR)	6,285/ 229	NR	NR	100	Hip, lower arm, upper arm	Calculated overall	1.45 (1.03-2.03)[7]
Holmberg 2006	Cohort[1]	Sweden	Malmö Preventive Project	NS	NR	F 11(NR) M 16 (NR)	33,346/ NR	3,915	NR	32	Hip, Forearm, Proximal Humerus, Ankle	Calculated overall	1.29 (0.54-3.13)[3]
Ivers, 2001	Cohort[1]	Australia	The Blue Montains Eye Study	NS	≥ 49	5	3,654/ 216	251	NR	57	Non-vertebral (exclude ribs)	Overall	0.90 (0.70-1.20)[4]
Jung, 2012	Cohort[2]	Korea	Eulji General Hospital out- patient clinic, Korea	NS	>20	5.7 (2.0)	2,282/ 1,268	81	NR, Korean	100	Non-vertebral (hip, distal radius, elsewhere)	Overall	1.62 (1.02-2.56)[3]
Keegan, 2002	Case-control	USA	Kaiser Permanente Medical	NS	≥45	Oct 1996	4,528/ 472	2,615	WHite 61% Asian 14.9%,	75	Foot, distal forearm, proximal humerus	Calculated overall	1.26 (0.87-1.83)[8]

Study, year	Study design	Country	Cohort name	DM type	Age (y)	Fol up y (SD)	Pop total/ DM	Fract	Ethnicity	F (%)	fracture sites included	Risk estimate's group	Risk estimate
			Centers in Northern California			May 2001			Black 12.7%, Hispanic 11.6%				
Kim, 2017	Cohort[2]	Korea	NHIS-KNHIS	T2D	≥50	6y	51,330/ 17,110	3,855	Korean	54	Non-vertebral	Female	1.14 (1.02–1.25)[3]
												Male	1.14 (0.93–1.39)[3]
Lee, 2015 (EPESE)	Cohort[1]	USA	EPESE	NS	≥ 65	6.5	2,704/ 566	572	Blacks 54.5% White 45% Others 0.5%	100	Hip and non-hip, non-vertebral	Hip fracture	1.27 (0.80–2.02)[9]
												Non-hip, non-vertebral fracture	1.23 (0.97–1.56)[9]
Napoli, 2014	Cohort[1]	USA	MrOS	NS	≥ 65	9.1 (2.7)	3,967/ 881	871	White 90% Black 4.07% Asian 3.19% Hispanic 2.10% Other 1.18%	0	Non-vertebral	Overall	1.12 (0.94-1.34)[10]
Rathmann 2015	Cohort[2]	Germany	German Disease Analyzer database	T2D	NR	2.9 (3.3)	598,208/ 299,104	11,535	NR	49	Hip, forearm, upper arm and shoulder	Calculated overall	1.41 (1.12-1.78)[11]
Schafer, 2010	Cohort[1]	USA	Health ABC study	NS	70-79	8.2 (2.3)	1,949/ 658	NR	White 58% Black 42%	50	Non-vertebral	Overall	1.42 (1.07–1.89)[12]
Schneider 2013	Cohort[1]	USA	ARIC Study	NS	45-64	Md 20	15,140/ 1,800	1,078	White 74% Black 26%	5	Hip, upper limb, lower limb	Calculated overall	1.78 (1.21-2.61)[13]
Schwartz, 2001	Cohort[1]	USA	SOF	T2D	≥ 65	9.4 (2.4)	9,654/ 657	2,624	"mainly white" (black women were excluded	100	Non-vertebral	Insulin user	1.58 (1.14–2.20)[4]
Schwartz, 2001												Non-insulin user	1.16 (0.99–1.37)[4]
Taylor, 2011	Cohort[2]	USA	5% random sample of Medicare	NS	≥ 65	4.2 p-y	1,694,051/ NR	124,241	White 88% Asian 1.3% African 7.8% Hispanic 1.5% Other 1.5%	58	Hip, distal radius/ulna, humerus, tibia/fibula	Calculated overall	1.13 (1.00-1.27)[14]

Study, year	Study design	Country	Cohort name	DM type	Age (y)	Fol up y (SD)	Pop total/ DM	Fract	Ethnicity	F (%)	fracture sites included	Risk estimate's group	Risk estimate
Wallander 2017	Cohort[1]	Sweden	FRAILCO	Both	≥65	Md 1.3	428,305/ 84,702	36,132	NR	58	Any (hip, wrist, upper arm, ankle, major osteoporotic- no vertebra included)	Calculated overall	1.13 (0.98-1.30)[6]

Fol up Follow-up; Frac fractures; F female; WHI-OS Women's Health Initiative- Observational Cohort; NHW - KNHIS Non-Hispanic white; F female; M male; NHIS- NSC National Health Insurance Service National Sample Cohort of the Korean National Health Insurance Service; EPESE North Carolina Established Populations for Epidemiologic Studies of the Elderly; ARIC The Atherosclerosis Risk in Communities Study; Md median SOF Study of Osteoporotic Fractures; p-y person-years; FRAILCO Fractures and Fall Injuries in the Elderly Cohort;

[1]Prospective [2]Retrospective

[3] Age adjusted, reported by gender

[4] Age and gender

[5] Age, gender, BMI, smoking, serum creatinine, visual acuity, falling frequency, lower limb disability

[6] Age, gender, height, weight

[7] Adjusted for baseline age, BMI group (<20, 20–30, >30), modified Charlson index, estrogen deficiency, MOF, prevalent rheumatoid arthritis, former osteoporosis treatment, glucocorticoid use >450 prednisone eq., family fracture history, current smoking, exercise level, prevalent alcohol related diagnoses

[8] Five-year age, gender, and race/ethnicity, as indicated by inpatient medical files (White, non-White, and unknown), and the following: age in years, self-reported race/ethnicity, and type of interview (in person vs. over the telephone).

[9] Age, race, BMI

[10] Adjusted for age, race, clinic

[11] Age, sex, diabetologist care, depression, chronic kidney disease, peripheral vascular disease, heart failure, hyperlipidemia, obesity

[12] Age, race, sex, clinic site, and total hip BMD

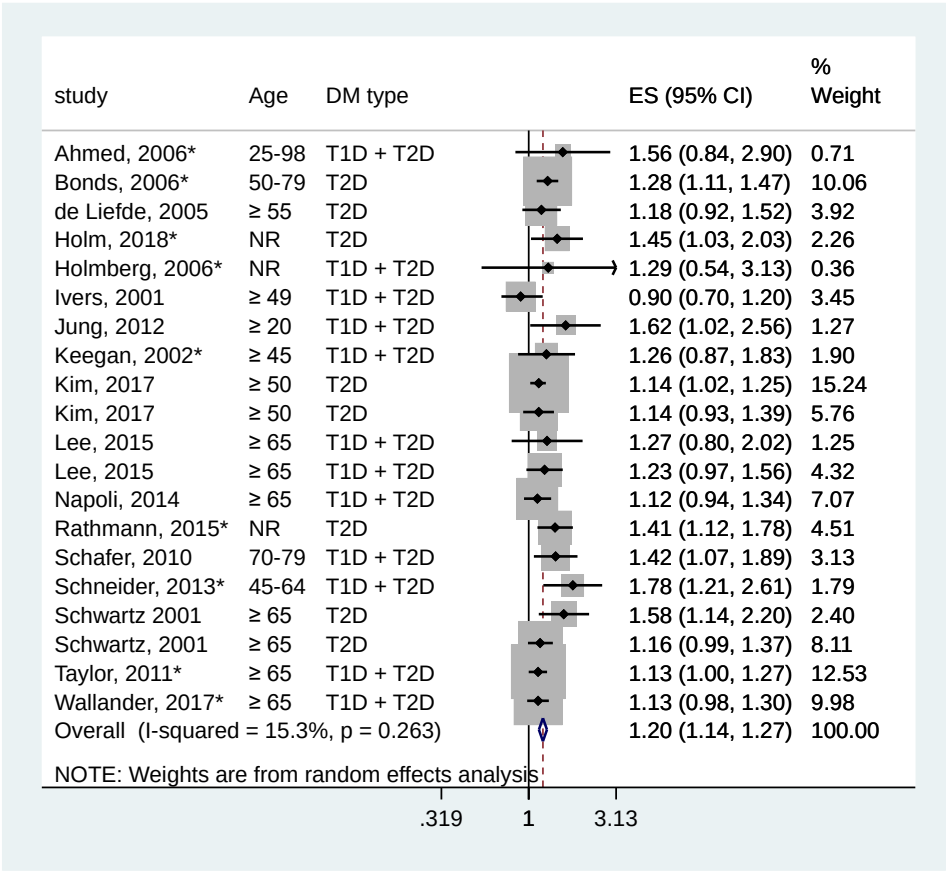
[13] Age, gender and race/study center, body mass index, sports-activity tertile, alcohol consumption, cigarette smoking, and medication use.

[14] Gender, race-ethnicity, age, calendar year, urban/rural, geographic location, median income, previous fracture, other predisposing conditions (glucocorticoid related, fall-related, renal disease, depressive illness, AMI, other heart disease, bone disease, cancer)

Non-vertebral fractures meta-analysis results

Overall risk

The risk of non-vertebral fractures was increased in diabetes (RR1.20 95%CI 1.14-1.27) and heterogeneity was not significant (I^2 15.3%, p=0.2) (Figure 3-16). Seventeen studies were included in this analysis, reporting data from 2,982,622 participants, 414,195 with diabetes and 185,363 fractures. Several subgroup analyses were anticipated in the protocol, however, due to the lack of data (as few studies reported specific subgroup risks), it was not possible to conduct age, DM type and BMI subgroup analyses.

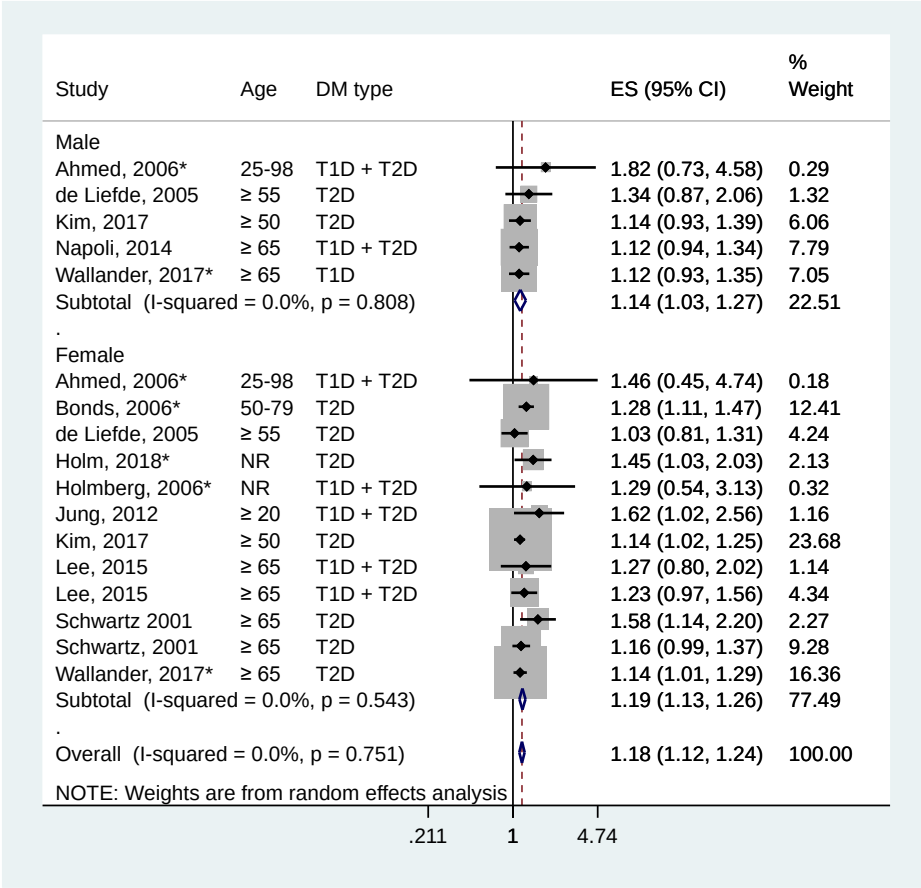


* Summarised using random-effects model

Figure 3-16 Forest plot overall risk of non-vertebral fractures in Diabetes

Subgroup analysis by gender

The risk of non-vertebral fractures was similar in male (RR 1.14, 95%CI1.03-1.27) and female (RR 1.19, 95%CI 1.13-1.26). No heterogeneity was observed (I^2 0.0% P=0.7) (Figure 3-17).

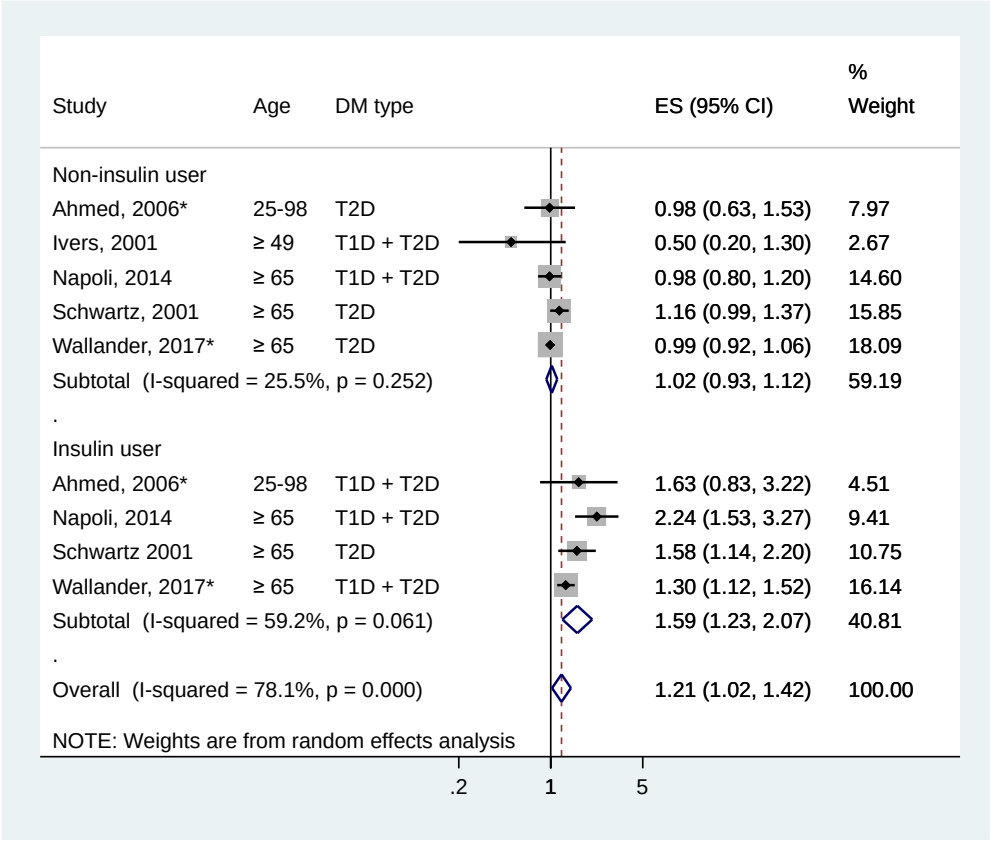


* Summarised using random-effects model

Figure 3-17 Forest plot risk of non-vertebral fractures in diabetes by gender

Subgroup analysis by Insulin use

The risk of non-vertebral fractures was higher in insulin users (RR1.59 95%CI 1.23-2.07) than in non-insulin users (RR1.02 95%CI 0.93-1.12). This analysis included eight studies. Heterogeneity was high (I^2 78.1%, p<0.001) (Figure 3-18).

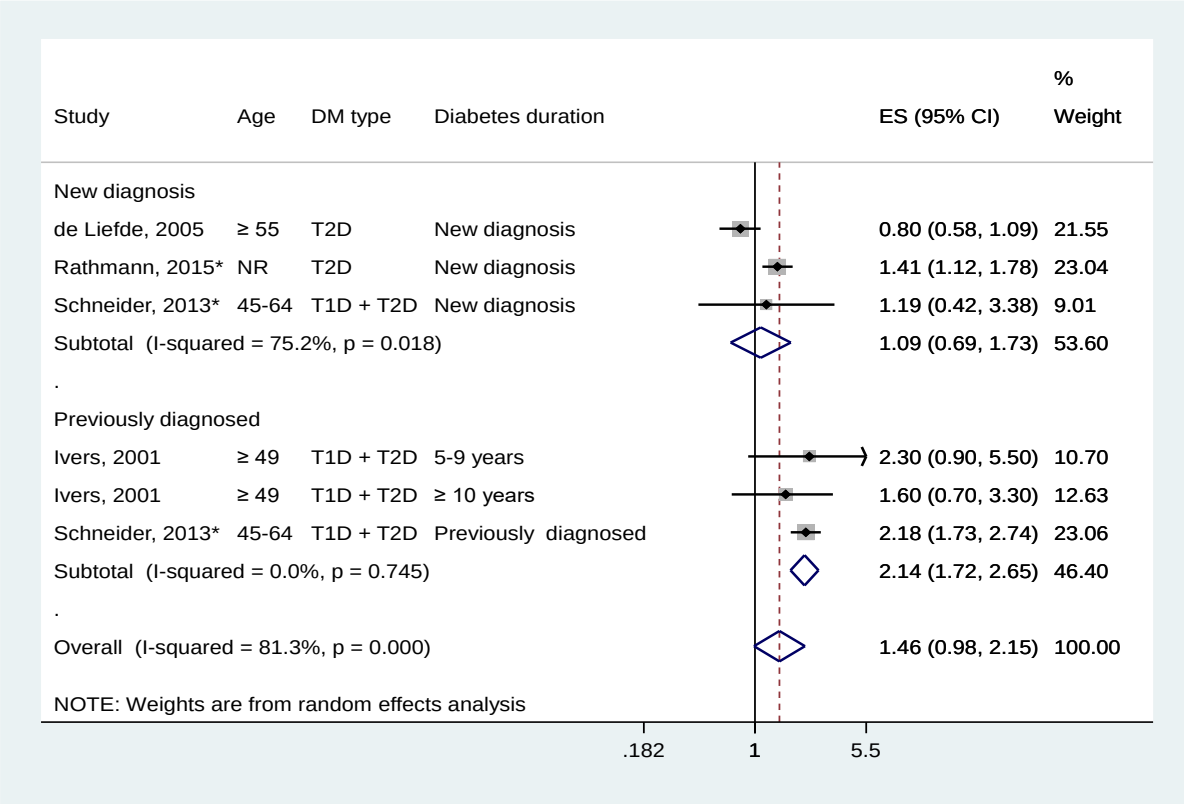


* Summarised using random-effects model

Figure 3-18 Forest plot risk of non-vertebral fractures in diabetes by insulin use

Subgroup analysis by DM duration

The risk of non-vertebral fractures was higher in previously diagnosed diabetes (RR 2.14 95%CI 1.72-2.65) than in newly diagnosed diabetes (RR1.09 95%CI 0.69-1.73), (RRR 0.51 95%CI 0.31-0.85). Four studies were included in this analysis and high heterogeneity was observed (I^2 81.3%, p<0.001) (Figure 3-19).

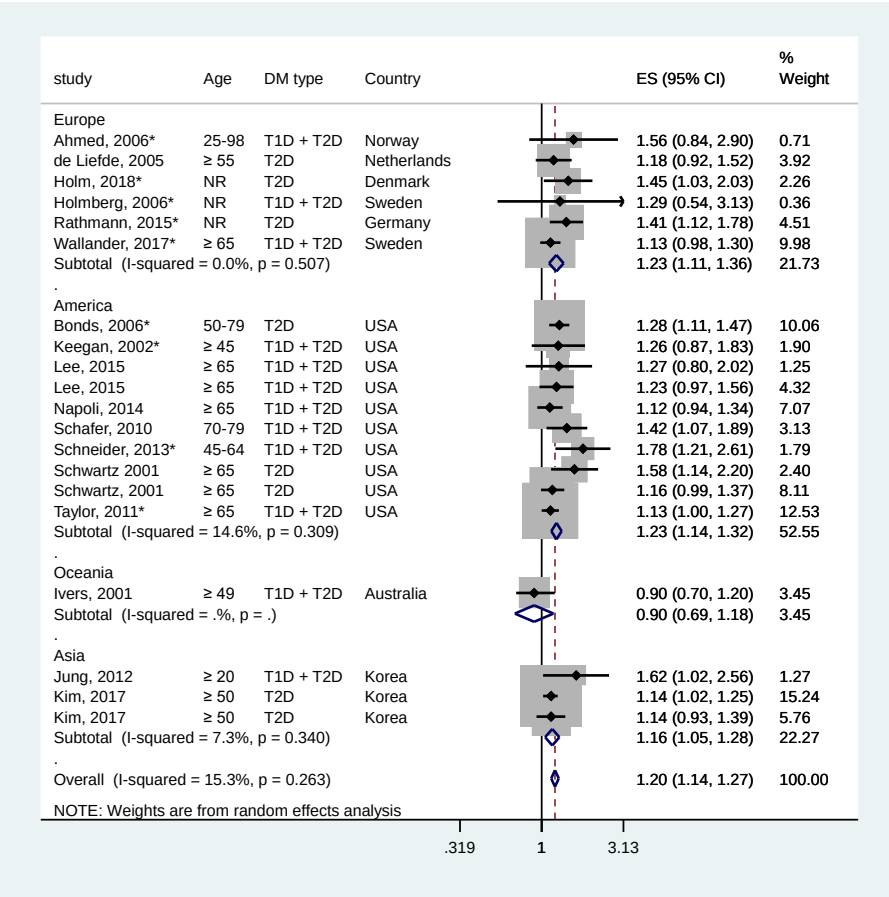


* Summarised using random-effects model

Figure 3-19 Forest plot risk of non-vertebral fractures in diabetes by DM duration

Subgroup analysis by geographical location

The analysis by geographical location showed similar RR for non-vertebral fractures in Europe (RR 1.23 95%CI 1.11-1.36), America (RR 1.23 95%CI 1.14-1.32) and Asia (RR 1.16 95%CI 1.05-1.28). The unique study from Oceania, an Australian study, reported a significantly lower RR (RR 0.90 95%CI 0.69-1.18) (Figure 3-20).



* Summarised using random-effects model

Figure 3-20 Forest plot risk of non-vertebral fractures in Diabetes by geographical location

Non-vertebral fractures sensitivity analysis

We ran the analyses excluding one study at a time and no important variation was observed in the results. The RR varied from RR 1.19, 95%CI 1.13-1.26 to RR 1.23, 95%CI 1.15-1.31 and the heterogeneity remained low, from 0.6% to 23.8%, suggesting that no study had an important individual impact in the results. In additional analysis, no important variation was observed when excluding the case-control study (RR 1.20, 95%CI 1.14-1.27) or the study from Australia (RR 1.20 95%CI 1.15-1.26). We also excluded each kind of risk estimate (i.e. RR, OR) with no important impact in the result, with the RR varying from RR 1.18, 95%CI 1.12-1.25 to RR 1.22, 95%CI 1.15-1.29.

Funnel plot

Visual analysis of the funnel plot suggested publication bias (Figure 3-21). The Egger's test resulted in p=0.013 and the Begg's test p=0.018 also suggestive of publication bias. As the heterogeneity was low and the studies had diverse sample sizes we applied the Trim and Fill

correction. The method removes small studies causing asymmetry, estimate the number of missing studies and add them and the estimates of their effect (147). Consequently, the method provides a RR for a symmetrical funnel plot, as if there was no publication bias. The Trim and Fill method included six hypothetical studies (Figure 3-22) and resulted in a RR 1.17, 95%CI 1.10-1.24, similar to the original results (RR 1.20 95%CI 1.14-1.27), suggesting that the publication bias did not have an important impact in the results.

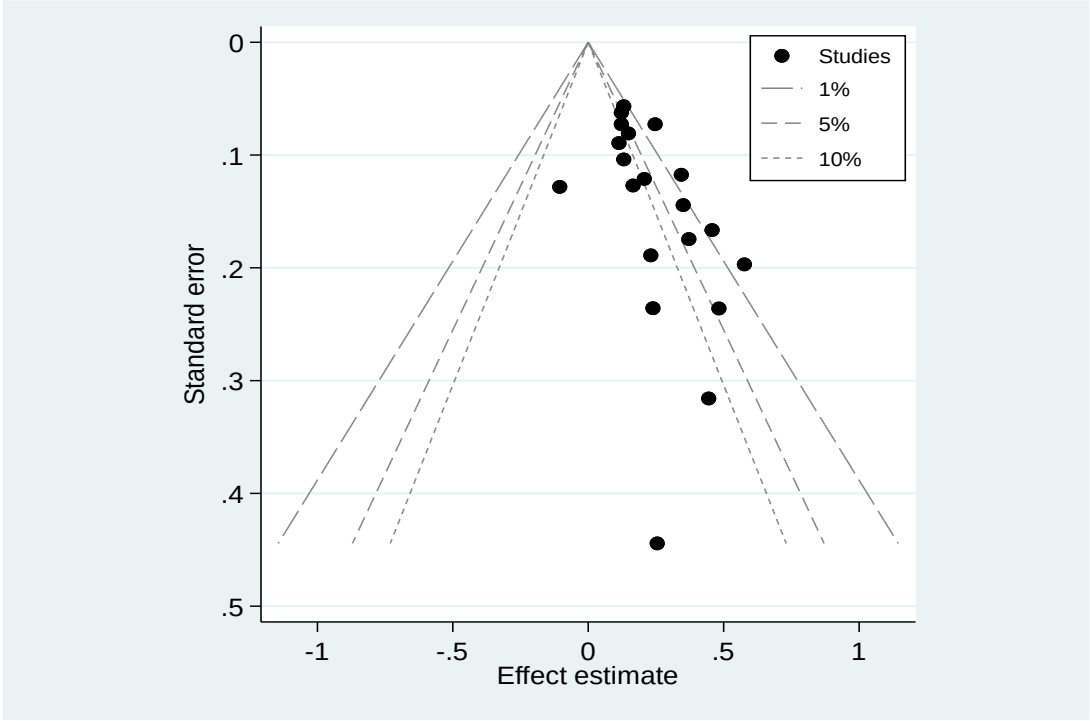


Figure 3-21 Funnel plot of the original studies included in non-vertebral fractures analysis

(1%, 5% and 10% shows the levels of significance)

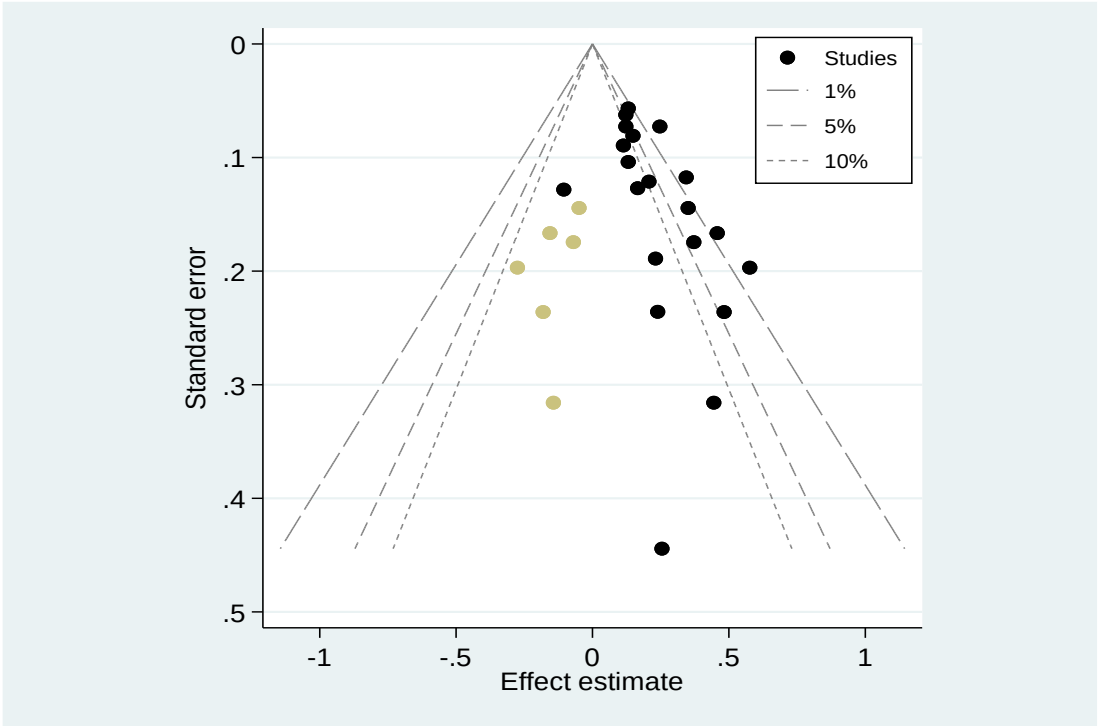


Figure 3-22 Funnel plot after Trim and Fill correction in non-vertebral fractures analysis.

The yellow dots are the included by the Trim and Fill method (1%, 5% and 10% shows the levels of significance).

Discussion

There is an increase in the risk of hip and non-vertebral fractures in diabetes, especially in insulin users. At the hip, the risk is higher in the younger population, females, T1D and those with longer disease duration.

Mechanism for increased risk of fracture

The increase in the risk of fractures in diabetes is multifactorial. Falls are probably a main feature. DM is associated with an increased risk of falls (93, 148, 149). Within the diabetic population, the risk of falls is higher in those in insulin use, those with MVD and in those with hypoglycaemic episodes (150-152). Bone features are also important. BMD can predict fractures in people with diabetes (20), however, on average, BMD is decreased in T1D and increased in T2D (84). Conversely, the risk of fractures is increased in both diabetes types, suggesting that BMD is not an important determinant of bone fragility in diabetes (153). These findings suggest that bone quality, rather than BMD, could be affected by diabetes.

Several studies have investigated bone microarchitecture in diabetes and the results are not consistent (25-28, 31, 32, 154). Neutral, favourable and unfavourable features have been

described in patients with diabetes compared to patients without diabetes (25-28, 31, 32, 154). The studies that showed favourable bone microarchitecture in the diabetes group reported data from cohorts with short diabetes duration and good metabolic control (31, 154). Conversely, Shanbogue reported unfavourable microarchitectural findings in participants with microvascular complications (MVD) both in T1D and T2D (25, 28). Nonetheless, studies that compared people with diabetes with and without fractures reported increased cortical porosity especially in the tibia in the group with previous fractures (29, 32). Although more data is needed to clarify how microarchitecture influence the risk of fractures in diabetes, so far, evidence suggest that the abnormalities in the cortical compartment, especially cortical porosity, mighyt be involved.

Bone strength in diabetes has also been investigated using reference point indentation. A small cohort of postmenopausal women with diabetes (n=19) showed that BMSi was 9.2% lower in the women with diabetes than in the controls and it was inversely associated with the duration of the disease (33). A population-based cohort study including 51 participants with T2D and 483 controls, also reported lower BMSi in the group with T2D, despite favourable aBMD and microarchitecture (31). These findings suggest that diabetes might affect bone material properties, independently of aBMD or bone microarchitecture.

Chronic hyperglycaemia favours non-enzymatic reactions between proteins and glucose producing AGEs. In animal models, the accumulation of AGEs affected bone material and biomechanical properties (155). Pentosidine is the most widely investigated AGE. The analysis of hip replacement samples has shown higher pentosidine content in trabecular and cortical bone of people with diabetes (156). In addition, serum and urinary pentosidine were associated with higher risk and prevalence of fractures (49, 157). These findings suggest that chronic hyperglycaemia might affect bone material properties.

Finally, antidiabetic drugs might also affect the risk of fractures in diabetes in several ways. *In vitro* evidence suggested an osteogenic effect of metformin and data from cohorts showed a neutral or positive effect on the risk of fractures (158-160). Sulfonylureas have no direct effect on bone but they were associated with an increase in the risk of fractures, probably due to hypoglycaemia episodes and falls (161). Conversely, data on incretin mimetics are inconsistent. A meta-analysis that investigated the risk of fractures associated with the use of glucagon like peptide 1 (GLP-1) reported a decrease in the risk of fractures with liraglutide, but an increase with exenatide (162). Furthermore, another meta-analysis that investigated

the effect of dipeptidil peptidase 4 (DPP-4) inhibitors on the risk of fractures reported a protective effect (163). However, these meta-analyses are based on data from adverse events reports in clinical trials and the results should be interpreted with caution. Thiazolidenediones increase adipogenesis and impair osteoblastogenesis and were associated with an increase in the risk of fractures (161, 164). More recently, the sodium/glucose co-transporter2 inhibitors (SGLT2) canaglifozin was also associated with an increase in the risk of fractures (165). However, empaglifozin and dapaglifozin seem not to affect bone metabolism (161). Insulin use was also associated with an increased risk of fractures (91, 93, 129, 160). This finding is probably associated with insulin indications in diabetes treatment and adverse effects, rather than with the actions of the drug. T1D patients are always treated with insulin. Furthermore, T2D patients using insulin are more likely to have diabetes complications and longer disease duration. Regardless of diabetes type, insulin users are more likely to be affected by hypoglycaemia than non-insulin users (161).

Comparison to prior studies

In 2007, two comprehensive meta-analyses investigated the risk of fractures in diabetes and reported similar results (10, 84); while the risk of hip fractures varied from 38 to 70% in T2D the risk reached a 6-fold increase in T1D. There was no increase in the risk of vertebral fractures. Since then, other meta-analyses have addressed the issue but in a less comprehensive approach. Fan et al investigated the risk of hip fracture, and also reported a higher risk in T1D (5-fold increase) than T2D (34% increase) (86). Dytfeld et al investigated the risk of hip and vertebral fractures in postmenopausal women with T2D and found a 26% increase in the risk of hip and no increase in the risk of vertebral fractures in this population (13). Conversely, Shah et al investigated the risk of fractures in T1D and reported a 3-fold increase in the risk of hip fractures, a 2-fold increase in the risk of spine fractures and a 3-fold increase in the risk of any fractures (14). Additionally, Vilaca et al investigated the risk of peripheral fractures in diabetes and reported an 30% increase in the risk of ankle fractures and a 15% decrease in the risk of wrist fractures (166). Recently, Wang et al investigated the risk of fractures in several sites assessing data from cohort studies. This study reported an increase in the risk of total, hip, upper arm and ankle fractures in diabetes but no increase in the risk at distal forearm and vertebra (85).

Our meta-analysis results are consistent with previous meta-analyses as we reported an overall 52% increase in the risk of hip fractures (10, 13, 84-86), with a significant 37% increase in the risk in T2D and a substantial 4 -fold increase in T1D. The risk in T1D is approximately double the risk observed in rheumatoid arthritis (RR 2.41 95%CI 1.83–3.17) and with glucocorticoid use (RR 2.01 95%CI 1.74–2.29) conditions known to affect the risk of fractures and for which guidelines recommend specific approaches (167, 168).

Burden of the disease

Although the magnitude of the increase in the risk of fractures is different in each diabetes type, we estimate that the increase in the number of fractures in both populations is clinically relevant. The increase in the risk is smaller in T2D, but around 90% of the diabetic population is affected by this type of the disease. Conversely, there is a 4-fold increase in the risk in T1D, but this corresponds to less than 10% of the diabetic populations. According to the Clinical Practice Research Datalink (CPRD), in the population older than 50 years the incidence of hip fractures in the UK was 22.4/10,000 person-year from 1988-2012 (169). Considering that the prevalence of diabetes in the UK is 7.7% and that 10% of this population is affected by T1D, in a population of 1,000,000 people, 7,700 would have T1D and would suffer 83 hip fractures in one year (76). The same number of people without diabetes would suffer 17 fractures at the same time. Furthermore, in this 1,000,000 population, 69,300 would be affected by T2D and would suffer 202 hip fractures, while the same number of people without diabetes, would suffer 155 fractures. These estimates showed that the number of fractures associated with T2D is higher (202 in T2D and 83 in T1D), but the excess of fractures is higher in T1D (47 in T2D and 66 in T1D; 40% higher in T1D). Regardless of diabetes type, there is an excess of fractures that brings additional burden to patients and healthcare systems.

Greater increase in fracture risk in T1D compared to T2D

The greater increase in the risk of hip fractures observed in T1D also agrees with all the previous meta-analyses that investigated the risk of hip fractures in both types of diabetes (10, 84-86). A number of features contribute to this finding. Firstly, insulin has an anabolic effect in bone and T1D is characterised by a lack of endogenous insulin. Although treatment aims to restore glucose homeostasis, it is possible that some degree of insulin deficiency could affect bone health. Secondly, T1D often is diagnosed in childhood or adolescence and might

compromise the peak of bone mass accrual. Bone mass is not affected in children with newly diagnosed diabetes (170), but bone accrual is compromised in children with poor glycaemic control as early as one year after diagnose (171). In addition, T1D children with fractures had poorer glycaemic control and lower total body BMD (172). A meta-analysis that assessed BMD in T1D reported significantly lower BMD at total body and femur in T1D despite gender or age. At the spine, BMD is decreased in female younger than 20 years old and any age males (17). These findings suggest that early onset T1D might compromise bone quantity and contribute to the substantial increase in the risk of fractures in this population. Finally, insulin treatment is associated with higher risk of hypoglycaemia and hypoglycaemic episodes are associated with higher risk of falls (173).

There were not enough data to perform a diabetes type subgroup analysis for non-vertebral fractures.

Subgroup analyses

In contrast with other studies, we reported a greater increase in the risk of hip fractures in women (77%) than in men (35%). This is probably a reflection of the greater number of studies included in this meta-analysis (n=37) compared with a maximum 25 studies in previous reviews (85). The bigger number of studies probably gave this analysis enough power to detect the difference. In the analysis of non-vertebral fractures, the risk was similar for men and women. As the number of studies involved in the non-vertebral fractures analysis is smaller (n=17) it is not possible to know if the effect of gender is different at each site or if the non-vertebral fractures analysis did not have enough power to detect an eventual small difference.

This is the first meta-analysis to assess the effect of age, insulin use, diabetes duration and BMI in the risk of fractures in diabetes. In the hip fractures analysis, we found a greater increase in the relative risk of fractures in people with diabetes younger than 65 years old, than in the population older than 65 years old. When stratified by age range, the risk was higher in younger age and decreased progressively to reach no increase in the risk in those older than 80. A number of features might contribute to this finding. Data from NHANES reported that the mean age of T2D diagnosis in 2000 was 46 years (174). Consequently, the younger population, especially population under 50 years old, is likely to include a higher proportion of T1D patients. The mean age of a hip fracture in general population is around 80

years-old (175, 176) and the absolute risk of fractures varies widely across the different age ranges. Data from the Clinical Practice Research Datalink (CPRD), a dataset representative of the UK population reported that the incidence of hip fractures from 1988-2012 in the population aged 18-49 years was 1.0 /10,000 person-year (1.4 /10,000 person-year in men and 0.6 /10,000 person-year in women). At 80-84 years old, the incidence was 70.0/10,000 person-year (40.1/10,000 person-year for men and 89.4/10,000 person-year for women) (169). The incidence of hip fractures in the younger than 65 years old is low (176). Considering that people with diabetes have an increase in the incidence of fractures, the impact on the relative risk will be greater when the basal incidence of fractures is lower, namely in a younger age-range (177). As the population at risk gets older, two concomitant phenomenon impact in the risk. On one side, as the population gets older and the background risk of fractures increases, the additional risk associated with diabetes play a less important role. In addition, diabetes is associated with a decrease in life expectancy. Estimates suggest that T2D decreases the life expectancy in 10 years and T1D decreases the life expectancy in 20 years. Estimates also suggest that half of the deaths associated with diabetes occurs before the age of 70 (75). Hence, we speculate that it is possible that the high mortality associated with diabetes would impact the risk of fractures in this disease, as some people with diabetes would not be at risk of hip fractures due to premature mortality (178).

Since meta-regression showed that age and diabetes type account for more than 80% of the variation in the risk of hip fractures in diabetes, we tried to conduct the subgroup analysis by age in each diabetes type. The number of studies was small, as not many studies reported the stratified risk estimates, but the risk seemed similar in T1D until the age of 70, when there is a decrease in the magnitude of the risk (Figure 3-8). As previously discussed, life expectancy is decreased in T1D and we speculate that premature mortality might have an impact in this risk. There were not enough data to perform the age range analysis in T2D.

The subgroup analysis by BMI showed no difference between the groups. The number of studies was small and the confidence intervals overlap, but the forest plot showed a trend to a decrease in the RR of hip fractures with the increase in BMI. It is known that obesity is protective against hip fractures (179). Mechanical and endocrine mechanisms lead to an increase in BMD and also fat has a cushion effect during the fall, protecting against the fracture (67). It is also known that obesity is highly prevalent in T2D. Data from the US reported that 85% of people with T2D are overweight or obese (180). Despite the high

prevalence of obesity in T2D, overall the risk of fractures is still increased in this population. The mechanisms that lead to the increase in the risk of fractures in diabetes are not fully elucidated so it is not possible to understand how these mechanisms interact with the mechanisms that decrease fractures in obesity.

There was a 59% increase in the risk of non-vertebral fractures and a 2-fold increase in the risk of hip fractures in insulin users. This risk is higher than the overall risk in diabetes and it is also higher than the risk in non-insulin users. This increased risk probably does not reflect an effect of insulin on bone but its indication and adverse effects. Insulin is the treatment used in T1D and data previously discussed suggest that this population has a greater increase in the risk of fractures within the population with diabetes. T2D can also be treated with insulin. However, in T2D, insulin is only used when other oral medications fail in achieving appropriate metabolic control. Consequently, T2D patients who use insulin are more likely to be affected by more severe diabetes, more likely to have diabetes for longer than non-insulin users and more likely to suffer from complications such as neuropathy, nephropathy and retinopathy. Retinopathy and neuropathy increase the risk of falls and nephropathy can lead to chronic kidney disease, which also increase the risk of fractures (173). In addition, people with diabetes treated with insulin have an increased risk of falls and fall related fractures (181). Lee et al, reporting data from a cohort with more than 650,000 male veterans older than 65 years, found an increase in the risk of fractures in T2D insulin users (160). Furthermore, there was a significant interaction with HbA1C levels. The increase in the risk of fractures in insulin users was greater in individuals with HbA1c < 6.5% suggesting that a tight glycaemic control might have adverse effect as it increases the risk of falls and consequently fractures (160). Conversely, Jensen, 2019, reporting data from a Danish database, showed that hypoglycaemia but not insulin was associated with an increase in the risk of fractures in T1D (182). In this study, based on registry data, 3% of all fractures in T1D were preceded by a hypoglycaemia episode. These findings suggest that hypoglycaemia and falls might play an important role in the risk of fractures associated with insulin use.

The subgroup analysis by diabetes duration showed a greater increase in the risk of fractures with longer diabetes duration both for hip and non-vertebral fractures. In the newly diagnosed group, probably there was not enough time for the harmful mechanisms associated with bone fragility in diabetes to act. In addition, in the T2D group, as obesity is the most important risk factor for T2D, the newly diagnosed diabetic population may be under

the protective effect of body weight excess on the risk of fractures. At the hip, the analyses showed a progressive increase in the risk with greater disease duration. People with longer disease duration have a longer exposure to hyperglycaemia and potentially harmful antidiabetic treatments. In addition, the likelihood of diabetic microvascular complications, and its harmful effects already discussed, also increases.

Heterogeneity

We found high heterogeneity in our analysis. Heterogeneity reflects the differences between studies (101). We included data from men and women, from 18 to 100 years old, with T1D and T2D so there was high clinical diversity. In addition, data came from prospective and retrospective cohorts and case-control studies, from recruited participants and registry data, adding substantial methodological diversity as well. Consequently, we expected that the effect size would vary between studies and to account for this variability, we used the random effects model in the analyses. We also explored this diversity using subgroup analysis, sensitivity analysis and meta-regression. These features should be considered while interpreting the results. Although we found a 52% increase in the risk of hip and a 20% increase in the risk of non-vertebral fracture in diabetes, this is an overall estimate. The risk will vary according to gender, age, diabetes type, diabetes duration and treatment. Although this is not the exact risk for a given patient, it is clinically useful to know that the risk is increased in people with diabetes and that this risk may vary, on dependence of individual characteristics.

Clinical approach to fracture prevention

The criteria to establish osteoporosis diagnosis in diabetes is the same as in the general population, based on the presence of fragility fractures and/or low BMD. BMD is one of the main tools used to predict the risk of fractures in clinical practice, however, BMD underestimates the risk of fractures in diabetes. Overall people with diabetes suffers a fracture with a BMD 0.5 standard deviation higher than people without diabetes (20). Furthermore, so far, FRAX, another fracture prediction tool used worldwide to estimate the risk of hip and major osteoporotic fractures, does not include the risk of fractures associated with diabetes in its calculation. Evidence suggest that FRAX also underestimates the risk of fractures in diabetes (20, 183). The IOF Bone and Diabetes Working group acknowledge these

evidences. A recent report suggested that patients with diabetes should be considered for treatment at more favourable FRAX and BMD values than patients without diabetes (159). The IOF Working Group report suggested that the BMD intervention threshold should be at a T-score of -2 at spine or hip in western populations (159). The current risk calculated by FRAX should also be adjusted. Some evidence suggested that having T2D is equivalent to adding 10 years of age or reducing the BMD T-score by 0.5 standard deviation (20). The Workgroup considers that the FRAX risk assessment should be adjusted for T2D and despite limitations, they recommend that risk associated with T2D should be substituted by the risk associated with rheumatoid arthritis in the current version of FRAX (159). In addition, patients with bone loss greater than 5% in two years should be considered for treatment especially when measurements are close to the intervention threshold.

There is no specific treatment for bone fragility in diabetes. As the risk of fractures seems to be associated with poor glycaemic control (144) and diabetic complications (64, 90), adequate metabolic control is advisable. However, the risk of hypoglycaemia should be considered, especially in the elderly. Two cohorts have assessed the relationship between the risk of fractures and metabolic control in elderly populations (160, 184). Lee et al assessed male veterans and reported an increase in the risk of fractures both at HbA1c< 6.5% and HbA1c> 9.0% (160). Conversely, Conway assessed a geriatric cohort and reported the lower risk of fractures in the group with HbA1c 6.5-6.9%, and no significant risk increase at other HbA1c groups (184). A tight metabolic control increases the risk of hypoglycaemic events. A number of studies have reported an increased risk of fractures associated with hypoglycaemia (173, 181, 182). Recently, the European Association for the Study of Diabetes (EASD) and the American Diabetes Society (ADA) guidelines have recommended a less strict glycaemic control in the elderly, to avoid hypoglycemic events and falls (185). Therefore, adequate metabolic control is the treatment target, but tight metabolic control should be avoided. In addition, antidiabetic medications with unfavourable effect on bone metabolism should be avoided in patients with diabetes and bone fragility (159).

So far, no trial was developed to assess specifically the efficacy of anti-osteoporotic medications in diabetes. The available data from existing studies showed similar effect in people with and without diabetes in regards to BMD increase and anti-fracture efficacy with alendronate, risendronate and teriparatide (186). Although in a *post-hoc* analysis of one trial, raloxifene was more effective in reducing vertebral fractures risk in T2D than in

postmenopausal women without diabetes, overall no difference was observed in the anti-fracture potential of raloxifene in the two groups. A systematic review in the topic concluded that diabetes did not seem to affect the fracture preventive potential of bisphosphonates (alendronate and risedronate), raloxifene or teriparatide (186). However, most of the data available assessed postmenopausal women with T2D and additional data about anti-fractures efficacy in other groups such as males and younger populations is required. Although denosumab is a potential treatment option, especially in the subgroup with impaired renal function, there is no specific data on the efficacy of denosumab in diabetes (159).

Strengths and weaknesses

This study has several strengths. This is the most comprehensive review on the risk of hip fractures, with the greater number of studies and most comprehensive subgroup analysis pooled so far. This is the first systematic review and meta-analysis on the risk of non-vertebral fractures in diabetes. A large number of studies were included and overall the quality of the studies was good. The high heterogeneity found in the hip fracture analysis was extensively explored by subgroup, sensitivity analysis and meta-regression.

However, this study also has limitations. This is a systematic review and meta-analysis update, so we relied on the search done by the previous systematic review (10). The initial study sifting, based on title and abstract was done by one reviewer but the random 10% double sifting kapa statistic for agreement was perfect. We could not investigate the effect of other features that affect the risk of fractures in diabetes such as BMD, falls and the competing risk of death. In addition, we could not investigate the effect of some features associated with diabetes such as metabolic control, the presence of microvascular complications, the effect of anti-diabetic drugs and hypoglycaemia in the risk of fractures.

Conclusion

This meta-analysis highlights the complexity of the assessment of the risk of fractures in diabetes. Although the mechanisms are not fully established, it is clear that people with T1D is the population at higher relative risk. Despite growing evidence on the increased risk of fractures in diabetes, the skeleton is not widely recognised as a site for diabetic complications. A review in diagnosis and management of bone fragility in diabetes by the IOF Bone and Diabetes Working group is the current guidance in how to manage these patients (159).

However, there is limited data on the assessment of fracture risk, the impact of the increased risk of fractures in DM management and the use of anti-osteoporotic treatments in this population.

Chapter 4

Discordant pattern of peripheral fractures in diabetes: A meta-analysis on the risk of wrist and ankle fractures

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This chapter was published as paper on Osteoporosis International Journal

Vilaca T, Walsh J, Eastell R Discordant pattern of peripheral fractures in diabetes: a meta-analysis on the risk of wrist and ankle fractures.

Osteoporos Int. 2019 Jan;30(1):135-143. doi: 10.1007/s00198-018-4717-0.

Introduction

There is an increase in the risk of hip fractures in diabetes, but the risk is less well described for other skeletal sites (10, 84). A number of cohorts have reported an increase in the risk of fractures in several sites in diabetes (139, 142, 152) . The Women's Health Initiative study showed an increased risk of any fracture and all the other fracture sites evaluated, except lower arm/wrist/hand in postmenopausal women (139). The majority of the participants were white, but other ethnic groups such as Black, Hispanic and minorities were also included (139). In elderly men, the risk of non-vertebral fracture is increased in models adjusted for age, race, clinic site and total hip BMD. However, in the further adjusted model, the risk remained increased only in insulin users (142). Diabetic men have higher BMD but lower bone strength and lower resistance to fractures (146). Conversely, a biracial cohort of diabetic elderly men and women reported a 64% increase in incident clinical fractures after adjustments for BMD and body composition features such as lean and fat mass, but no additional risk was associated with insulin use (152). Several small studies have reported different findings, but meta-analyses have agreed that there is an increase in the risk of hip fracture, in T1D and T2D (10, 12-14, 84). Results are less consistent for other skeletal sites. The risk of any fracture is increased in T1D (14) and T2D (10, 84). Shah et al. reported an increase in the risk of vertebral fractures in T1D (14), but there was no significant increase in T2D (10, 13, 84). Previous evaluation of specific sites such as the distal forearm, ankle, proximal humerus in T2D showed significant increase only at the foot (10). More data are required to establish the site-specific risk of fractures in this population.

The increase in the risk of fractures in diabetes is not directly associated with BMD (84). The risk of fractures is increased in T1D and T2D, but BMD is decreased in T1D, and increased in T2D (17, 84, 144). The increased risk of fractures in T1D is greater than would be expected for the decrease in BMD, suggesting that other features (such as bone quality, increased fall risk or altered biomechanics) might play a role. Despite increased BMD in T2DM, BMD is still able to predict fracture risk, but for a given T-score, people with diabetes have a higher risk of fractures than people without the disease (20). Conversely, for a similar fracture risk, women and men with diabetes have a higher BMD than people without the disease (20, 142). Therefore, BMD does not fully reflect bone fragility in diabetes.

Diabetes and osteoporosis are both major public health concerns. In 2015, the global prevalence of diabetes was 8.8%, and estimates suggest that it will reach 10.4% in 2040 (1). Type 2 diabetes (T2D) is the most common form of the disease, accounting for 90% of cases. Osteoporosis is estimated to cause 9 million fractures annually worldwide, resulting in significant disability (187). Both diseases affect mainly the elderly, their prevalence is increasing worldwide, and both are associated with significant morbidity and mortality (1, 187). As life expectancy is increasing, the prevalence of both diabetes and osteoporosis is expected to rise, increasing the burden for health care systems.

Although hip fracture risk is increased in patients with diabetes, the mechanisms associated with bone fragility in this population are not established. Investigations so far suggest that accumulation of advanced glycation end-products (AGEs) and low bone turnover may impair bone material quality (188). Microarchitectural assessments have identified structural abnormalities (188). High-resolution peripheral computed tomography (HR-pQCT) was used to evaluate microarchitecture at the ankle and the wrist. Several studies reported a decrease in volumetric bone mineral density (vBMD) and an increase of cortical porosity (25-29). To evaluate if there is a clinical consequence for these microarchitectural abnormalities, we performed a systematic review and meta-analysis of the risk of ankle and wrist fractures in diabetes.

Methods

PRISMA-P was used to develop the protocol, and PRISMA statement was used as a guidance (189). One reviewer searched databases like Medline, EMBASE and LILACS in March 2017. “Diabetes mellitus”, “fracture”, “ankle”, “wrist”, “radius” and “forearm” were used in the

research. There were no limits in regards to languages or date of publication. In order to capture all the available information, studies that reported the risk of fractures in adults (>18 years) with diabetes (type 1 and type 2) compared with healthy controls were included.

Additionally, we reviewed references from relevant published papers. Studies were excluded if they included children, had unclear diabetes diagnosis criteria, did not have a comparison group without diabetes or if it was not possible to extract or calculate the relative risk for fractures.

We extracted the data using a piloted questionnaire in Google Forms. For each study, data on the first author's name, country, year of publication, study design and name, source of funding, source and age of population, numbers of exposed and unexposed subjects, numbers of events in each group, follow-up period (in cohorts), type of diabetes, gender, risk estimates and corresponding confidence intervals, possible confounders, and factors controlled for by multivariable analysis were extracted. If the relative risk was not reported, but there were enough data for adequate calculations, the risk was calculated using standard formulas.

The Newcastle-Ottawa quality assessment tool was used (190). Specific questionnaires were applied for cohorts and case-control studies. We used funnel-plots to evaluate publication bias.

The studies were grouped in meta-analyses, using the random-effects model. Adjusted relative risks controlling for potential confounders such as age, gender and race were combined using Stata version 14 (Stata corporation, College Station, Texas). As just a few studies adjusted the risk for weight or body mass index (BMI) and BMD, which were potentially the most important confounders, these adjustments were excluded.

Results

The research process is summarised in Figure 4-1. Initial electronic searches resulted in 756 citations. After the evaluation of inclusion and exclusion criteria, eleven articles were selected, six with data about ankle fractures (distal tibia and fibula) and ten about wrist fractures (distal radius and ulna). Data were described as relative risk in cohorts and odds ratio in case-control studies. As the frequency of fractures is low, odds ratio and relative risk can be assumed as reporting similar risk estimates (191).

The Newcastle-Ottawa toll was used to evaluate the studies' quality. The studies had good quality and the scores varies from 6 to 8 (out of 9). Funnel plot evaluation revealed no publication bias.

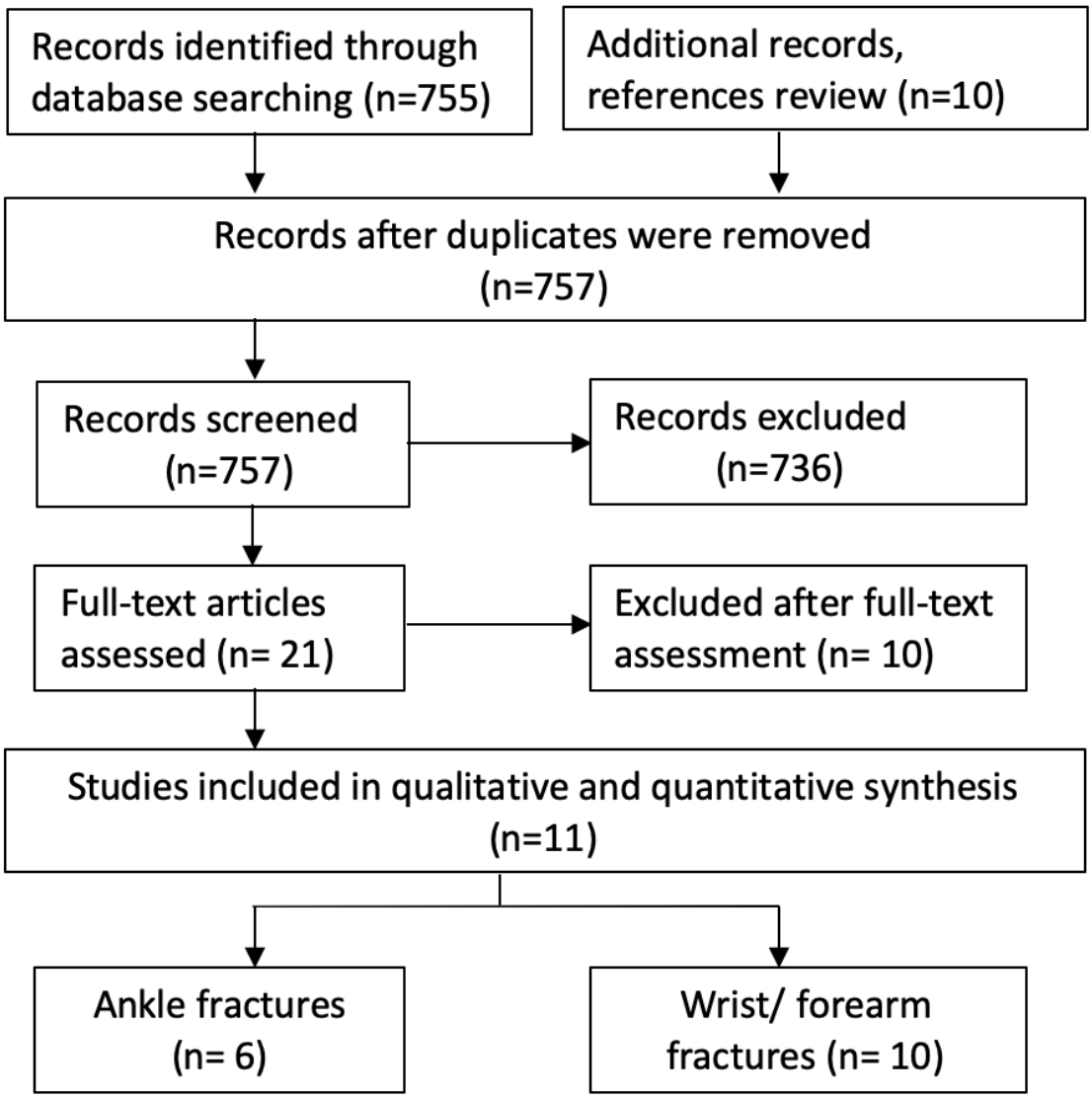


Figure 4-1 Research process flowchart (adapted from PRISMA diagram)

Ankle fractures

For ankle fractures, six studies were selected: five cohorts (115, 116, 137, 192) and one case-control study (193). In the cohorts, the mean follow-up was 7.4 years (range 1.3 to 25 y). In the case-control study, for each case, three controls were selected (193). The age range was from 27 to 109 years. One study recruited participants from 27 to 61 years (115), another one

from 49 to 97 years (116). Pritchard et al. evaluated registry data from people older than 50 years (193). The remaining three studies report data from people older than 65 years (93, 137, 192). Two studies reported data from the United States (137, 192), one from Canada (193), two from Sweden (93, 115) and one from Australia (116). Most of the studies reported data from white populations (93, 115, 116, 193). Where other ethnicities were included, less than 10% were black people and 2% were other ethnicities (92, 137). Three studies reported data from a registry (93, 137, 193) and three recruited participants (115, 116, 192). Two studies reported data only from T2D (93, 192), while the others did not state the disease type (115, 116, 137, 193). Three studies excluded fractures associated with high-energy/trauma (115, 192, 193), while the three others (registry based), did not report the energy associated with the fractures (93, 116, 137). Two studies reported data just from women (192, 193), two reported the risk specifically for men and women (93, 115) and the two others did not report gender-specific risk (116, 137). In the three studies which reported BMI, it was on average 10% higher in the group with diabetes (93, 192, 193). In three studies, there was a significant increase in the risk of ankle fractures in diabetes [RR 1.28 (CI 1.12- 1.47) (93); RR 1.34 (CI 1.30-1.39) (137); RR 3.36 (1.58-7.15) for women (115)]. In the other three studies, the increase in the risk of ankle fractures in diabetes was not statistically significant [RR1.1 (CI 0.6-1.9)(116); RR 1.14 (0.93-1.38) (193); RR 1.22(0.76-1.97)(192). Two studies reported a higher risk in the insulin-user group RR 2.35 (1.04-5.28) (192) and RR 1.47 (1.24-1.76) (93).

Data were summarised in a meta-analysis. Two studies are reported as unadjusted data (193) (93), the remaining data were adjusted for age (115, 192), age and gender (116), gender, race, age, calendar year, urban/rural area, geographic region and median income (137). The studies characteristics are listed on Table 4.1.

When the data were pooled together, we found an increased risk of ankle fractures in people with diabetes (RR 1.30 95% CI 1.15-1.48) (Figure 4-2). Subgroup analysis found a higher risk in people with diabetes who used insulin (RR 1.56 95% CI 1.15 – 2.12) than the risk in non-insulin users (1.24 95% CI 1.07-1.45). The meta-analysis summarises data from 2,137,223 participants and 15,395 fractures.

Table 4.1 Ankle fracture study characteristics

First author	Year	Country of study	Cohort name	Study design	Follow up (cohort) y	Number of participants	Source of population	Age y (mean ± SD)	RR (95% CI)	DM diagno	Fracture diagnosis	DM type
Ivers	2001	Australia	The Blue Mountains Eye Study	Cohort study	5 years	DM 216 Non-DM 3,438	Recruitment	49-97 (66)	1.1 (0.6-1.9)	Self-report	Self-report confirmed by radiology report	NR
Schwartz	2001	United States America	Study of Osteoporotic Fractures (SOF)	Cohort study	9.4 y (mean)	T2D 657 Non-DM 8,997	Recruitment	> 65 (71 ±5)	NI 1.22 (0.76-1.97) I 2.35 (1.04-5.28)	Self-report	Self-report confirmed by radiology report	T2D
Holmberg	2006	Sweden	Malmo Preventive Project	Cohort study	M (7-25) 19y W (7-22) 15y	DM 381 Non-DM 32,738	Recruitment	27-61 (M 44 W 48)	M 0.73 (0.23-2.29) W 3.36 (1.58-7.15)	Self-report	Registry	NR
Taylor	2011	United States America	Random 5% sample of Medicare beneficiaries form 2000-2005	Cohort study	4.2	Total pop 1,694,051 (DM non-reported)	Registry	>65 y	1.35 (1.30-1.39)	Registry	Registry	NR
Pritchard	2011	Canada	Manitoba Bone Density Program	Case-control	[#]Case-control 3 controls/ each case	DM 3,054 Non- DM 9,151	Registry	> 50 y 68(±9)	1.14 (0.93-1.38)	Medical records	Registry	NR
Wallander	2017	Sweden	FRAILCO	Cohort study	1.3 y (mean)	T1D 2,883 T2D 79,159	Registry	> 65 y (80.2 ±8.2)	NI 1.10 (0.9 – 1.33)	Registry	Registry	T1D and T2D

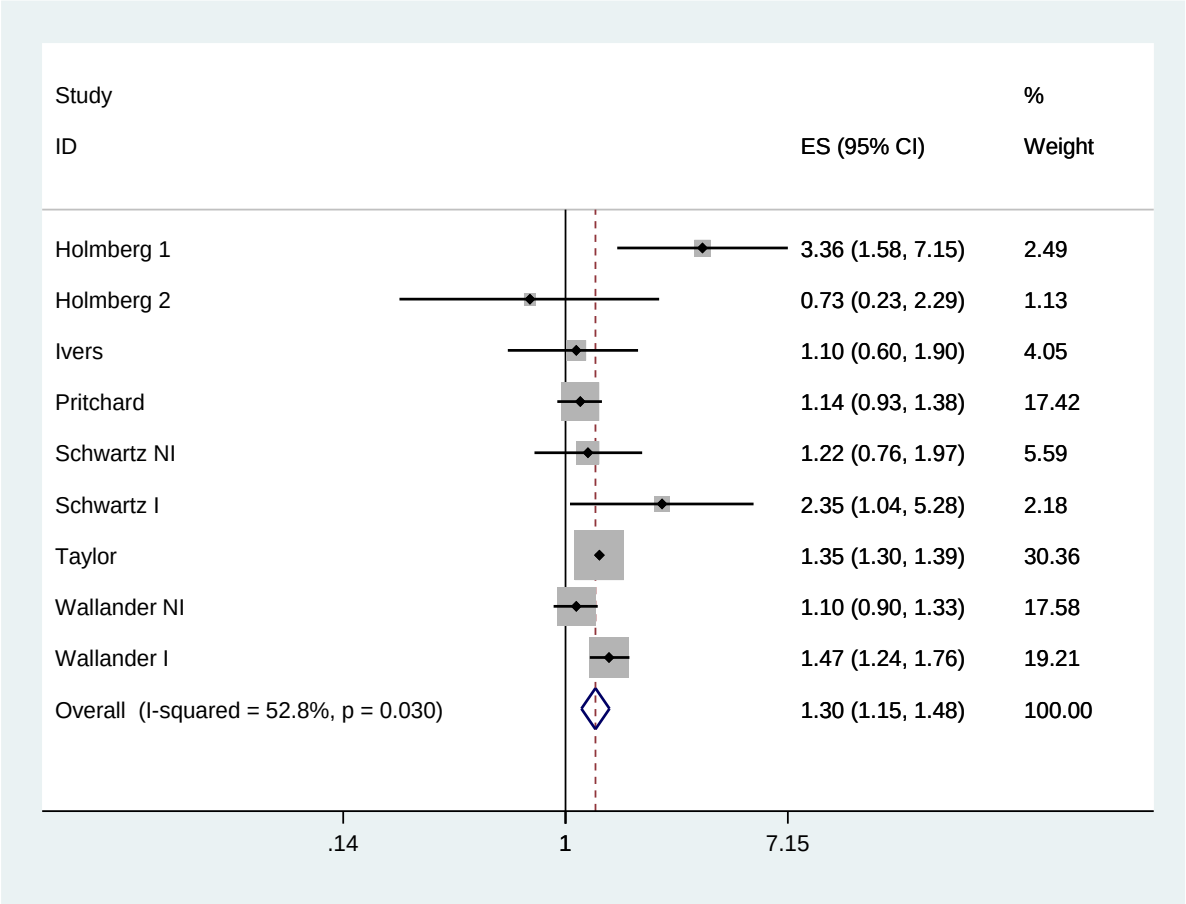
First author	Year	Country of study	Cohort name	Study design	Follow up (cohort) y	Number of participants	Source of population	Age y (mean ± SD)	RR (95% CI)	DM diagno	Fracture diagnosis	DM type
						Non-DM 343,603			I 1.47 (1.24 – 1.76)			

Table 4.2 Wrist fracture study characteristics

First author	Year	Country of study	Setting - Name of the study/cohort	Study design	Follow up (cohort) y (years)	Number of participants	Source of population	Age y (mean)	RR (95% CI)	DM diagnosis	Fracture diagnosis	DM type
Schwartz	2001	United States America	Study of Osteoporotic Fractures (SOF)	Cohort study	9.4 y (mean)	T2D 657 Non-DM 8,997	Recruitment	> 65 (71 ±5)	NI 0.83 (0.56-1.22) I 1.43 (0.71-2.88)	Self-report	Self-report confirmed by radiology report	T2D
Ivers	2001	Australia	The Blue Mountains Eye Study	Cohort study	5 y	DM 216 Non-DM 3,438	Recruitment	49-97 (66)	0.7 (0.2-2.3)	Self-report	Self-report confirmed by radiology report	NR
Keegan	2002	United States of America	Kaiser Permanent Northern California	Case-control		Cases: 1000 Controls: 1913 Number of DM not reported	Recruitment	>45y	0.88 (0.68-1.16)	Self-report	Registry	NR

First author	Year	Country of study	Setting - Name of the study/cohort	Study design	Follow up (cohort) y (years)	Number of participants	Source of population	Age y (mean)	RR (95% CI)	DM diagnosis	Fracture diagnosis	DM type
Leslie	2005	Canada	The First Nation Cohort	Retrospective cohort study	12 y	DM 3,699 Non-DM 107,578	Registry	> 50 y (68±9)	0.86 (0.65-1.15)	Medical records	Registry	NR
De Liefde	2005	Netherlands	The Rotterdam study	Cohort study	6.8 y	T2D 792 Non-T2D 7,191	Recruitment	>65	1.4 (0.81 - 2.41)	Test result	Registry	T2D
Gerdhem	2005	Sweden	Osteoporotic Prospective risk assessment (OPRA)	Cohort study	3 – 6.5 (4.6) y	DM 67 Non-DM 961	Recruitment	>75 y	0.74 (0.45-1.21)	Self-report	Self-report + registry	NR
Holmberg	2006	Sweden	Malmo Preventive Project	Cohort study	M (7-25) 19y W (7-22) 15y	DM 381 Non-DM 32,738	Recruitment	27-61 (M 44 W 48)	M 0.46 (0.21- 1.04) W 0.73 (0.38- 1.41)	Self-report	Registry	NR
Taylor	2011	United States America	Random 5% sample of Medicare beneficiaries form 2000-2005	Cohort study	4.2	Non-DM pop 1,694,051 (DM pop not reported)	Registry	>65 y	0.95 (0.93-0.98)	Registry	Registry	NR
Harness	2012	United States America	Kaiser Permanent South California	Retrospective cohort study	6 y	DM120,796 Non-DM 403,816	Registry	>60 y	1.05 (0.99-1.1)	Registry	Registry	NR
Wallander	2017	Sweden	FRAILCO	Cohort study	1.3 y (mean)	T1D 2,883 T2D 79,159	Registry	> 65 y (80.2 ±8.2)	NI 0.65 (0.55-0.77)	Registry	Registry	T1D and T2D

First author	Year	Country of study	Setting - Name of the study/cohort	Study design	Follow up y (cohort) (years)	Number of participants	Source of population	Age y (mean)	RR (95% CI)	DM diagnosis	Fracture diagnosis	DM type
						Non-DM 343,603			I 0.68 (0.58-0.81)			



Holmberg 1 women; Holmberg 2 men; NI non-insulin users; I insulin users;

Figure 4-2 Forest plot risk of ankle fractures in diabetes

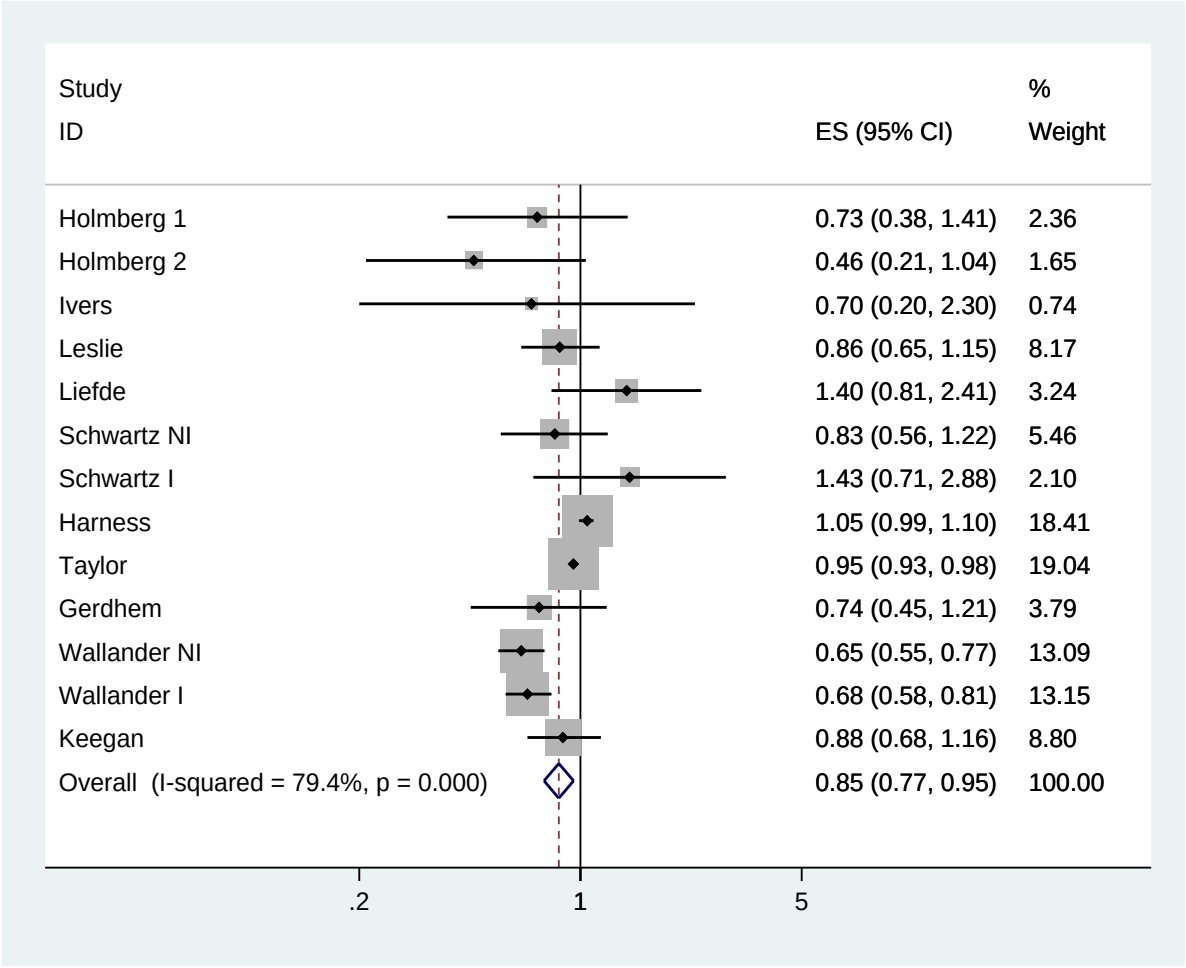
Wrist fracture

Of the ten wrist fracture studies selected, nine were cohorts, six prospective (41, 90, 93, 115, 116, 192) and three retrospective (137, 194, 195), and one study was a case-control (141). The follow-up ranged from 1.3 to 25 years, and the mean was 7.6 years in the cohorts. Four studies reported data from the United States (137, 141, 192, 195), three from Sweden (41, 93, 115), one from the Netherlands (90), one from Canada (194) and one from Australia (116). Most studies reported data from white populations (41, 90, 93, 115, 116) while the North American ones included other ethnicities. One study included Canadian indigenous people (194), and two others included around 10% of Black people (137, 192). In one study, 20% were Asiatic, 15% Black and

10% Hispanic (141). In another, 66% were non-white (195), although it was not specified which ethnicities was included, due to non-availability of the data in the registry. In six studies participants were recruited (41, 90, 115, 116, 141, 192) and in four studies data came from a registry (93, 137, 194, 195). The age of the participants varied from 20 to 109 years. Two studies reported data from young people, one from people older than 20 years (194) and the other from 27 to 61 years (115). Two studies reported data from the fifth decade, one study observed people older than 45 years (141) and another older than 49 years (116). One study reported data from people older than 55 years (90) and another one older than 60 years (195). Four studies reported data from elderly people, three from 65 years (93, 137, 192) and one from people older than 75 years (41). Three studies reported the risk for men and women (90, 93, 115), two reported data just from women (41, 192) and the other five did not state gender-specific risks (116, 137, 141, 194, 195). The majority of the studies did not specify the type of diabetes, although three reported data just from T2D (90, 93, 192). Two studies reported the specific risk in insulin-users (93, 192). In two studies, high-energy fractures were excluded from the analyses (115, 192), while the others made no distinction. Two studies reported a significant decrease in the risk of wrist fractures: Taylor et al. reported RR 0.95 (95%CI 0.93-0.98) (137) and Wallander et al. reported RR 0.67 (95%CI 0.59-0.76) (93). In all the other studies the association was not significant: RR 0.73 (95%CI 0.38-1.41) for women and RR 0.46 (95%CI 0.21-1.04) for men (115); RR 0.70 (95%CI 0.20-2.30) (116); RR 0.86 (95%CI 0.65 – 1.15) (194); RR 1.40 (95%CI 0.81-2.41) (90), RR 0.83 (95%CI 0.56-1.22) for non-insulin-users and RR 1.43 (95%CI 0.71-2.88) for insulin-users (192); RR 1.05 (95%CI 0.99 – 1.10) (195); RR 0.74 (95%CI 0.45 – 1.21) (41); and OR 0.88 (0.68- 1.16) (141). The studies characteristics are described on Table 4.2.

All the studies were pooled in a meta-analysis. We found a significant decrease in the risk of wrist fractures [RR0.85 (95% CI 0.77 – 0.95)] (Figure 4-3). The risk was not decreased in insulin-users [RR 0.91 (95% CI 0.45-1.85)]. The analysis included data adjusted for age (90, 115, 192), age and gender (116), gender, race, age, calendar year, urban/rural area, geographic region, median income (137), age, gender and ethnicity (141) and unadjusted data (41, 93, 194, 195). However, sensitivity analysis of adjusted and unadjusted data showed similar patterns. This meta-analysis

reports data from 2,773,222 subjects and 39,738 fractures. The studies included men and women, from 20 to 109 years, the vast majority with type 2 diabetes.



Holmberg 1 women; Holmberg 2 men; NI non-insulin users; I insulin users;

Figure 4-3 Forest plot risk of wrist fractures in diabetes

Discussion

There is an increase in the risk of ankle fractures, and a decrease in the risk of wrist fractures in diabetes. Ankle and wrist fractures have distinct epidemiological patterns. Ankle fractures are not considered typical osteoporotic fractures (196, 197). Having an ankle fracture is a predictor of a future fracture at other sites (197). However, the risk of an ankle fracture is not associated with low axial BMD, but with increased weight and BMI (196, 197). Overweight and obesity are

highly prevalent in T2D, the main group evaluated in this study (1). Interestingly, microarchitecture abnormalities without decreased BMD were previously associated with ankle fractures (198). Stein et all reported disrupted microarchitecture but no BMD abnormalities in postmenopausal women with ankle fractures (198). Microarchitectural abnormalities were observed mainly in the trabecular compartment. These abnormalities were more pronounced at the tibia, but also observed at the wrist. The authors argue that this finding could highlight underlying bone fragility despite relatively normal BMD. Cortical porosity was not associated with ankle fractures in this study (198).

Conversely, wrist fractures are a major osteoporotic fracture. They account for up to 18% of all fractures in people older than 65 years, and can be the first clinical indicator of osteoporosis (199). Bone density, geometry, microstructure and strength are all determinants of wrist fractures (200). Melton et al reported microarchitectural abnormalities in a wrist fracture population, and the deficit in trabecular bone was relatively greater than in cortical bone. Cortical porosity was similar in cases and controls and some analyses suggested that Colles' fractures are associated with disruptions of trabecular architecture (200). Some evidence suggests that obesity decreases the risk of wrist fractures (201).

The discordant pattern observed in this study might reflect the weight excess in the population observed. Although obesity is generally considered protective against fracture, the effect on fracture risk is site-dependent (188, 201). Several studies reported a decrease in the risk for femoral and wrist fractures and an increase for ankle and upper arm fractures (201). Obese adults have greater BMD than normal weight controls (67). Evans et al. reported favourable microarchitecture features such as increased cortical and trabecular BMD in obese people when compared to normal-weight adults (67). Non-bone features also play an important role. The thick soft tissue has a protective effect in absorbing the impact in hip fractures (201). Obese people tend to fall backwards or sideways, which might favour the occurrence of upper arm fractures over wrist fractures (201). On the other hand, an increase in the mechanical strain at the ankle has been reported (196).

Hyperglycaemia is present in both T1D and T2D, but the pathophysiology of each type is different. T1D is characterised by insulin deficiency that often starts before the peak of bone mass accrual.

On the other hand, in T2D there is insulin resistance which starts most frequently in adulthood, although a trend for a precocious start has been observed recently. Obesity is also more frequent in T2D than T1D, although the prevalence of obesity in T1D has been rising, especially associated with intensive insulin therapy (202). These features contribute to the BMD pattern observed in diabetes. BMD is decreased in T1D and increased in T2D (84). In a meta-analysis that evaluated BMD in both types of diabetes, BMI was significantly associated with BMD in T2D but not in T1D (84). How all these different features impact in the risk of fractures is still to be defined. The increase in the risk is remarkably higher in T1D, but successive meta-analyses have described a progressive lower risk: from RR = 6.94 (95% CI 3.25-14.78) by Vestergaard in 2007 to RR=3.78 (95% CI2.05-6.98) by Shah in 2015 (14, 84). The absolute risk in T2D is lower than T1D but 90% of people with diabetes have T2D, and there are estimates for an increase of T2D prevalence worldwide (1). This suggests that the majority of fractures associated with diabetes will affect the T2D population.

Several studies have described microarchitecture in T2D, and non-favourable findings are observed in the cortical compartment (26-28, 31, 32). Two studies have reported an increase in cortical porosity at the radius (26, 28) and two others at the tibia (27, 32). Besides, the standard ultradistal site, Nilsson et al evaluated a more proximal section, located at 14% of the limb length (a site of mainly cortical bone) and found a decrease in cortical porosity at the radius (31). The diabetic groups evaluated are diverse, including people with different ages, disease duration and complications. All these features could contribute to the non-consistent findings and make difficult to establish a more specific pattern for the cortical compartment findings in this population.

The fracture pattern observed in this study is similar to the pattern described in obesity, despite different microarchitectural findings in both diseases. In diabetes, the described pattern is a decrease in volumetric bone mineral density (vBMD) and an increase in cortical porosity (25-29). In obesity, Evans et al reported greater vBMD and lower cortical porosity (67). These findings suggest that microarchitecture is not the main determinant of peripheral fractures in these populations.

This study has limitations. A major limitation is the combination of type 1 and type 2 diabetes in the same analysis. T1D is associated with the lack of insulin and T2D with insulin resistance, with different consequences to bone health. However, few studies addressed specifically each type of the disease, preventing this analysis. Although men, non-white and T1D participants were included they account for the minority of the groups and this should be taken in account while evaluating the results. The majority of the participants were white postmenopausal women, a group especially susceptible to fractures. A large amount of data came from registry studies, which do not specify the population characteristics, and many potential confounders such as age, weight/BMI, type, duration and age of onset of diabetes, metabolic control, and the presence or absence of microvascular complications could not be addressed. Different factors were used to adjust the risk estimates in each study. Consequently, unadjusted data and data adjusted for different factors were used in this analysis. As just a few studies reported data adjusted for weight, these adjustments were excluded.

High heterogeneity was found in both analyses. Heterogeneity is a measure of the variability between studies. This review included data from cohort and case-control studies, from recruited participants and registry data so methodological variability is expected. In addition, data from T1D and T2D was collected and the age range varied from 27 to > 90 years old so clinical variability was also expected. These data should be considered while interpreting the results. For ankle fractures analysis, the risk of fracture is increased by 30% in diabetes, but this risk is an overall estimate that might vary according to age, diabetes type and other features. Conversely, the risk of wrist fractures was decreased by 15% in diabetes, but this estimate might also vary in individual settings.

It was desirable to pool together data from studies with adjustments for weight/ BMI and to compare them with the unadjusted ones to investigate the role of BMI in the association between diabetes and ankle/wrist fractures. However, this comparison was not possible as just two studies had this adjustment for the risk of ankle fractures (92, 193) and three for wrist fractures (90, 92, 93). The evaluation of data adjusted and unadjusted for weight could help to elucidate the amount that obesity contributes to fracture risk in diabetes.

Therefore, there are still important questions to be answered for bone health in diabetes. The site-specific pattern of fractures in people with diabetes is still being established. Also, investigations of the effect of many features, such as the type, age of onset and duration of the disease, the metabolic control and diabetic complications are ongoing. More information about the pattern of fractures in diabetes and how these individual features affect the risk of fractures in this population might help to understand how the diabetes affects the skeleton. This is important for planning management and designing adequate interventions.

In summary, there is an increase in the risk of ankle fractures and a decrease in the risk of wrist fractures in diabetes, despite adverse microarchitectural properties at both sites. Obesity, which is considered protective against the most fractures, but increases the risk of ankle fractures, may play a role in these fractures pattern in diabetes, independently from microarchitecture. More studies are needed to clarify the features associated with the increased risk of fractures in the diabetic population to guide adequate management.

Chapter 5

Bone density, structure and strength in diabetes

Chapter 5 Bone density, structure and strength in diabetes

Background

Diabetes and aBMD

A number of studies have assessed aBMD in diabetes (17)(84, 171). Two meta-analyses have shown an increase in aBMD in T2D and a decrease in T1D (17, 84). This disparity might be associated with differences on the age of onset, pathophysiology of the disease and presence of comorbidities, such as obesity, as previously discussed (chapter 3). In T1D, the early onset might compromise peak bone mass accrual and hypoinsulinemia might affect bone quantity (17).

Diabetes and bone microarchitecture

Several studies have investigated bone microarchitecture in T2D and the results are conflicting, as previously discussed in chapter 1. Most of the studies were conducted in T2D and there is substantial design variability. Despite inconsistent data, these findings suggest that bone microstructure might be affected by diabetes. Several individual characteristics, such as sex, age, BMI, BMD and also features associated with diabetes such as age of onset, disease duration, severity, treatment, metabolic control and the presence of complications might influence bone microarchitecture. All these features could have affected the results and contributed to the conflicting findings.

In T1D, data are rather scarce and only one previous study assessed T1D. Shanbhogue et al recruited 55 T1D patients, 29 without microvascular disease (MVD-) and 26 with microvascular disease (MVD+). The groups were compared to age, gender and height matched controls and also to each other. The study did not find significant differences in the comparison between MVD- and controls. However, when comparing MVD+ and controls, MVD+ had lower trabecular and cortical vBMD at the radius and also lower vBMD and lower cortical thickness at the tibia. There were no differences in cortical porosity. When comparing MVD+ and MVD -, MVD+ patients had lower vBMD, Tb.vBMD, Tb.Th at both the radius and tibia. At the tibia, there was lower cortical area, higher Tb.Sp and trabecular network inhomogeneity. All the differences persisted after

adjusting for potential covariates (ie, age, BMI, gender, menopausal status in women, duration of disease, and glycemic control). Noteworthy, MVD+ had lower total hip BMD than MVD- (25). These results suggest an impact of MVD on bone microarchitecture.

Evidence in microarchitecture in diabetes, both type 1 and type 2 is not consistent. Most of the data come from studies in patients with T2D. In these studies, the most frequent finding was in cortical porosity (26-29, 32). Nilsson et al has investigated an additional site, the 14% length site in T2D and found an intriguing decrease in cortical porosity at this site (31). In order to investigate the cortical compartment more extensively in T1D we decided to add the assessment of the 14% site to this project.

T1D and bone strength

In T1D, Shanbhogue reported several unfavorable findings in MVD + compared to MVD- and these deficits resulted in lower estimates of bone strength (both total stiffness and failure load). However, there was no difference when comparing people with diabetes and matched controls (25).

This conflicting evidence suggests that additional studies need to be done to assess bone strength in diabetes and also the effect of MVD.

Background summary

Despite conflicting evidence in microarchitecture and bone strength analysis in diabetes, several meta-analyses in literature and previous chapters in this thesis reported an increase in the risk of fractures in diabetes, especially in T1D. The previous study that assessed bone microarchitecture in T1D reported no difference between diabetes and matched control groups but unfavourable findings while comparing T1D MVD+ with controls and T1D MVD-. This evidence suggests a role for microvascular complications, but there are limited data addressing the issue.

In addition, previous evidence has also shown that T1D is the group affected by the higher risk of fractures. Therefore, we speculated that determinants of the increase in the risk of fractures will be more evident in T1D than in T2D and we conducted a study investigating bone microarchitecture in T1D.

Research question and hypothesis

Main research question:

- Is there an impact of T1D on bone microarchitecture and is this influenced by the presence of neuropathy?

Secondary research questions:

- Is there an impact of T1D on bone mineral density (BMD) and is this influenced by the presence of neuropathy?

Hypothesis

Our hypothesis is that there is an impairment in bone microarchitecture in T1D and that the presence of neuropathy could influence it.

Methods

Participants

Participants were recruited according to the inclusion criteria detailed in chapter 2. Participants with T1D were evaluated for the presence of neuropathy and categorised in type 1 diabetes with neuropathy (T1DN+) and type 1 diabetes without neuropathy (T1DN-) groups, as previously described in chapter 2. Individuals without diabetes were recruited as controls.

DXA was used to measure lumbar spine and total hip aBMD (Discovery A, Hologic Inc., Bedford, MA, USA). HR-pQCT was used to assess bone geometry and microstructure at the distal radius and distal tibia (XtremeCT, Scanco Medical AG, Zurich, Switzerland) (chapter 2). Microarchitecture was assessed at the standard site and at 14% bone length site (chapter 2).

Micro finite element analysis was used to estimate bone strength at standard and 14% sites at the radius and tibia (version 1.13; FE-solver included in the Image Processing Language, Scanco Medical AG, Zurich, Switzerland) (Chapter 2).

Statistical analysis

The three groups' demographics were compared using the ANOVA test. For characteristics only relevant to the groups with diabetes, independent t-test was used. Results are described as mean and standard deviations (SD).

Variables were tested for normal distribution using Kolmogorov- Smirnov test. Logarithmic transformation was tried but it did not result in normal distribution, especially for bone turnover markers. Since not all the variables had normal distribution, the Kruskal Wallis test was used in the analysis. Variables are described as median and interquartile range. For these analyses $p<0.05$ was considered significant.

When the test reported a significant difference between the three groups, the Mann Whitney test was applied in each pair of groups to investigate the difference. As multiple tests increase the risk of false positive the Bonferroni correction was considered, resulting in a p value of 0.017. Analyses were performed using IBM SPSS Statistics for Mac (Version 25.0. Armonk, NY: IBM Corp.).

Results

Sixty participants were recruited; 20 participants T1DN+, 20 T1DN- and 20 healthy controls (control). Groups were matched by gender resulting in 8 female trios and 12 male trios. Individual matching for age, height and weight was not possible, but there were no significant differences between the groups on these features. The population characteristics are described in Table 5.1

Table 5.1 Characteristics of the study population Mean (SD)

	T1DN+	**T1DN-**	**Control**	**p**
N	20	20	20	
Age (y)	47.7 (11.0)	49.6 (13.1)	49.1 (12.5)	0.872
Height (cm)	172.6 (8.2)	171.4 (10.3)	170.6 (9.7)	0.792
Weight (kg)	77.6 (18.4)	72.9 (12.0)	71.4 (10.7)	0.358
BMI (kg/m^2)	25.9 (5.2)	24.8 (3.6)	24.4 (2.5)	0.486
HbA1c	70.2 (14.3)	62.5 (14.6)	34.7 (3.2)	<0.001
Diabetes duration	28.9 (10.6)	24.1 (15.3)	NA	0.108
HESC[1]	54.7 (20.3)	78.2 (7.9)	NA	<0.001

	T1DN+	T1DN-	Control	p
FESC[1]	58.1 (25.6)	86.5 (4.9)	NA	<0.00
TCNS	13.3 (5.7)	2.5 (1.7)	NA	<0.001
DPN sural right conduction velocity[2]	32.1 (17.9)	48.4 (4.2)	NA	<0.001
DPN sural right amplitude[3]	3.6 (2.3)	11.7 (6.3)	NA	<0.001
DPN sural left conduction velocity [4]	36.5 (23.4)	49.8 (5.8)	NA	0.02
DPN sural left amplitude[5]	3.6 (4.0)	10.4 (4.9)	NA	0.001

HESC Hands electrochemical skin conductance; FESC Feet electrochemical skin conductance; TCNS Toronto Neuropathy Clinical Score

[1] n=19 for T1DN+ [2]n=14 for T1DN+; n=19 T1DN- [3]n=17 for T1DN+; n=19 T1DN- [4]n=8 for T1DN+; n=19 T1DN- [5]n=11 for T1DN+; n=19 T1DN-

aBMD results

All participants were assessed at the hip and lumbar spine (LS). Bone mineral density, bone mineral content and each site areas were not different for lumbar spine (LS), femoral neck (FN) or total hip (TH) in the three groups (Table 5.2) (**Error! Reference source not found.**-**Error! Reference source not found.**).

Table 5.2 aBMD results for T1DN+, T1DN- and control median (IQR)

	T1DN+	T1DN-	Control	p value
L1-L4 AREA	61.7 (59.2,69.6)	63.7 (58.5,73.6)	65.6 (59.8,74.5)	0.622
L1-L4 BMC	65.1 (53.9,75.5)	61.9 (52.3,79.8)	67.6 (50.3,74.2)	0.977
L1-L4 BMD	1.0 (0.9,1.1)	1.0 (0.9,1.1)	1.0 (0.8,1.0)	0.605
LS T-score	-0.3 (-1.7,0.5)	-0.7 (-1.7,0.1)	-0.8 (-2.0, -0.2)	0.597
LS Z-score	0.3 (,1.7,1.5)	-0.5 (-1.2,1.1)	-0.5 (-0.9,0.3)	0.759
FN AREA	5.5 (5.0,6.0)	5.4 (5.0,5.8)	5.1 (4.9,5.8)	0.49
FN BMC	4.0 (3.4,4.9)	4.4 (3.7,5.1)	4.0 (3.5,4.6)	0.376
FN BMD	0.8 (0.6,0.8)	0.8 (0.7,0.9)	0.8 (0.7,0.8)	0.185
FN T-score	-1.2 (-2.0,-0.5)	-0.7 (-1.5,0.0)	-1.2 (-1.6,-0.6)	0.181
FN Z-score	-0.2 (-1.2,0.4)	0.0 (,0.6,0.7)	,0.4 (,0.6,0.1)	0.151
TH area	42.9 (36.4,47.5)	40.9 (35.5,46.4)	40.8 (34.7,45.5)	0.814
TH BMC	37.5 (31.8,46.5)	39.2 (32.8,48.3)	37.7 (30.2,44.0)	0.782
TH BMD	0.9 (0.8,1.0)	1.0 (0.9,1.0)	0.9 (0.8,1.0)	0.518
TH T-score	-0.5 (-0.9,0.2)	-0.2 (-0.7,0.6)	-0.6 (-1.1,0.0)	0.423
TH Z-score	0.1 (-0.7,0.6)	0.2 (-0.4,1.1)	-0.1 (-0.4,0.3)	0.488

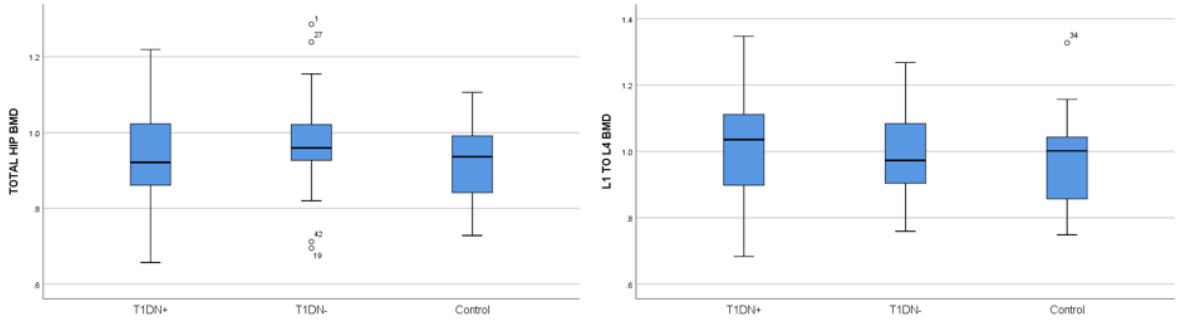


Figure 5-1 DXA a BMD A Total Hip B Lumbar Spine

HR-pQCT results

All the participants were assessed at the wrist and ankle. Nine standard radial scans were excluded due to movement artefacts (4 in the T1DN+ group, 4 in the T1DN- group and 1 in the control group). Nineteen 14% length radius site scans were not available due to technical issues (movement artefact or the 14% site was out of the scanning area - Figure 5-2). One 14% length tibia was not included due to movement artefacts.

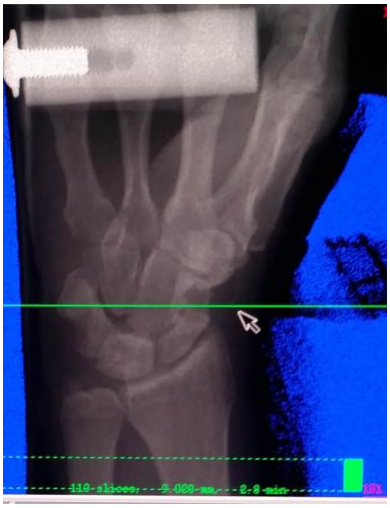


Figure 5-2 HR-pQCT 14% site scanning site could not be placed inside the scanning area

Standard site

At the standard site, in the analysis of the three groups, there was a significant difference in tibial cortical porosity (p= 0.028) and tibial connectivity (p=0.002) (Table 5.3). The comparison

between the groups has shown that T1DN+ participants had greater tibial cortical porosity (56% higher, p=0.009) (Figure 5-3) and greater tibial connectivity than T1DN- (125% higher, p=0.001) (Figure 5-4). Tibial connectivity was also greater in T1DN+ than in controls (80% higher, p=0.002). There were no significant differences between the groups in other tibial or radial features.

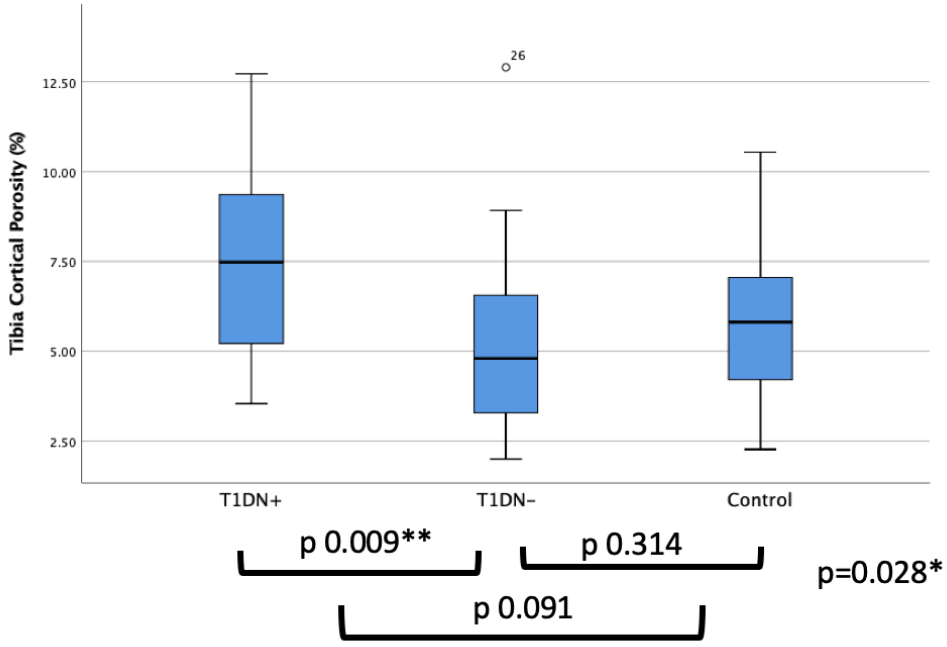
Table 5.3 HR-pQCT standard site results Median (IQR)

	T1DN+	T1DN-	Control	p value
Tibia				
Total Area (mm^2) Tt.Ar	853.3 (705.3, 939.4)	743.9 (673.6, 861.4)	783.8 (602.0, 854.5)	0.249
Cortical area (mm^2) Ct.Ar	131.0 (106.4, 144.2)	144.1 (110.0, 165.9)	133.1 (114.2, 158.9)	0.695
Trabecular area (mm^2) Tb.Ar	696.4 (592.4, 792.0)	611.1 (567.1, 673.0)	644.6 (480.3, 701.7)	0.196
Total density (mg HA/cm3)	303.6 (267.3, 330.5)	331.5 (273.8, 357.0)	300.4 (282.1, 330.1)	0.292
Cortical density (mg HA/cm3) Ct.BMD	845.9 (817.0, 895.1)	901.5 (861.0, 929.4)	892.2 (832.5, 916.0)	0.106
Cortical thickness Ct.Th	1.2 (0.9, 1.3)	1.2 (1.0, 1.5)	1.2 (1.1, 1.4)	0.643
Cortical perimeter	114.6 (103.9, 121.7)	106.6 (102.7, 117.7)	110.3 (96.6, 117.1)	0.459
Trabecular density (mgHA/cm3)	189.9 (165.4, 204.6)	199.0 (156.0, 213.0)	167.6 (150.1, 193.4)	0.224
Meta trabecular density (mgHA/cm3)	250.1 (224.2, 264.0)	255.7 (207.4, 271.0)	234.8 (217.2, 251.4)	0.39
Inner trabecular density (mgHA/cm3)	150.3 (121.5, 167.9)	156.4 (120.5, 176.8)	123.8 (103.2, 151.0)	0.213
Meta/Inn trabecular density (no units)	1.7 (1.5, 1.8)	1.7 (1.5, 1.9)	1.9 (1.6, 2.1)	0.251
Trabecular BV/TV (no units)	0.2 (0.1, 0.2)	0.2 (0.1, 0.2)	0.1 (0.1, 0.2)	0.225
Trabecular number	2.1 (1.6, 2.3)	1.9 (1.7, 2.2)	1.7 (1.6, 2.0)	0.251
Trabecular thickness	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.895
Trabecular separation	0.4 (0.4, 0.5)	0.4 (0.4, 0.5)	0.5 (0.4, 0.5)	0.288

	T1DN+	T1DN-	Control	p value
Trabecular inhomogeneity	0.2 (0.2, 0.2)	0.2 (0.2, 0.2)	0.2 (0.2, 0.3)	0.126
Connectivity Density	0.9 (0.7, 1.4)	0.4 (0.3, 0.7)	0.5 (0.4, 0.9)	0.002*
Cortical tissue mineral density (mg/cm3) Ct.TMD	1006.6 (988.2, 1040.5)	1031.1 (997.9, 1054.9)	1025.6 (985.8, 1040.8)	0.376
Cortical porosity (%) Ct.Po	7.48 (5.19, 9.48)	4.8 (3.1, 6.64)	5.81 (4.14, 7.36)	0.028*
T Mean cortical pore diameter (mm) Ct.Po.V	0.2 (0.2, 0.2)	0.2 (0.2, 0.2)	0.2 (0.2, 0.2)	0.777
SD of mean cortical pore diameter (mm) Ct.Po.Dm.SD	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.874
Periosteal perimeter (mm)	118.1 (109.6, 127.8)	109.0 (104.1, 117.9)	113.1 (96.7, 120.5)	0.106
Endosteal perimeter (mm)	115.6 (104.2, 127.7)	111.0 (99.9, 125.9)	117.2 (101.0, 122.3)	0.587
Stiffness (S in kN/mm)	233.9 (193.5, 288.3)	238.9 (202.5, 306.0)	231.9 (188.4, 273.4)	0.713
Estimated failure load (F.ult in kN]	11.7 (9.6, 14.7)	12.1 (10.2, 15.4)	11.7 (9.4, 13.6)	0.698
% trab distal load (%)	62.4 (57.8, 69.6)	57.1 (54.7, 66.3)	59.9 (52.3, 64.4)	0.37
% trab proximal load (%)	39.4 (34.6, 48.3)	34.5 (32.4, 42.1)	35.6 (31.9, 39.9)	0.287
Trab average von Mises stress (Tb.VM, in MPa)	6.1 (5.6, 6.5)	6.1 (5.9, 6.5)	6.2 (6.0, 6.4)	0.849
Cort average von Mises stress (C.VM in MPa)	8.6 (8.3, 8.8)	8.7 (8.6, 8.9)	8.7 (8.5, 8.8)	0.112
Cortical area fraction (calculated) (Ct.Ar/Tt.Ar)	0.2 (0.1, 0.2)	0.2 (0.2, 0.2)	0.2 (0.2, 0.2)	0.314
Cortical area fraction (calculated) (Ct.Ar/Tt.Ar)*100	15.8 (12.5, 19.3)	17.4 (15.1, 23.3)	18.6 (15.7, 19.6)	0.314
Radius				
Total Area (cm2)	361.8 (301.6, 420.4)	345.9 (278.1, 424.3)	333.5 (274.6, 403.1)	0.84
Cortical area (cm2)	66.8 (53.4, 73.3)	68.1 (49.4, 73.8)	52.4 (46.5, 71.1)	0.516

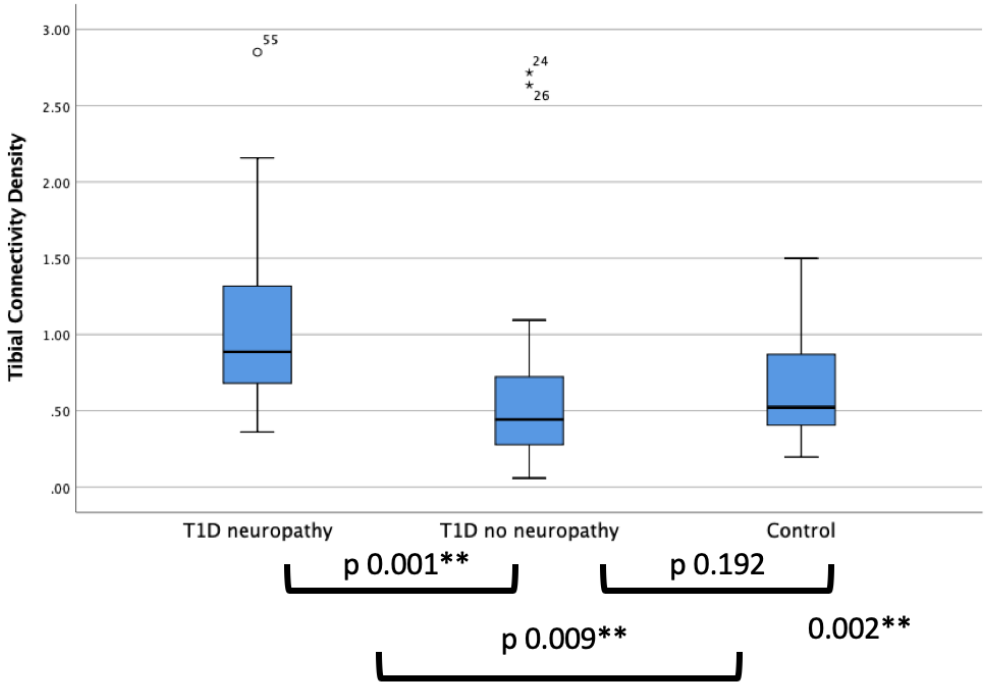
	T1DN+	T1DN-	Control	p value
Trabecular area (cm2)	297.2 (240.2, 344.0)	281.0 (204.3, 358.1)	258.6 (224.3, 349.4)	0.925
Total density (mg HA/cm3)	299.7 (287.9, 357.2)	326.3 (292.7, 360.4)	312.9 (265.1, 351.0)	0.678
Cortical density (mg HA/cm3)	873.8 (842.4, 895.8)	876.8 (850.4, 904.8)	872.6 (816.2, 895.2)	0.577
Cortical thickness	0.8 (0.7, 0.9)	0.8 (0.6, 0.9)	0.7 (0.6, 0.9)	0.336
Cortical perimeter	82.3 (74.6, 89.9)	79.3 (72.9, 87.9)	79.1 (70.5, 84.2)	0.739
Trabecular density (mgHA/cm3)	169.1 (139.1, 203.6)	173.5 (152.5, 198.0)	173.7 (136.5, 192.1)	0.931
Meta trabecular density (mgHA/cm3)	214.5 (204.6, 261.8)	219.8 (202.5, 252.2)	230.0 (198.9, 238.1)	0.968
Inner trabecular density (mgHA/cm3)	137.9 (93.0, 173.6)	135.1 (117.6, 177.6)	139.0 (92.5, 152.8)	0.91
Meta/Inn trabecular density (no units)	1.6 (1.5, 2.2)	1.7 (1.5, 1.8)	1.7 (1.5, 2.0)	0.841
Trabecular BV/TV (no unts)	0.1 (0.1, 0.2)	0.1 (0.1, 0.2)	0.1 (0.1, 0.2)	0.931
Trabecular number	1.9 (1.7, 2.2)	2.0 (1.8, 2.1)	2.0 (1.9, 2.1)	0.604
Trabecular thickness	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.656
Trabecular separation	0.4 (0.4, 0.5)	0.4 (0.4, 0.5)	0.4 (0.4, 0.5)	0.814
Trabecular inhomogeneity	0.2 (0.2, 0.2)	0.2 (0.2, 0.2)	0.2 (0.2, 0.2)	0.65
Connectivity Density	0.6 (0.4, 0.8)	0.4 (0.2, 0.6)	0.6 (0.4, 0.9)	0.101
Cortical tissue mineral density (mg/cm3)	1020.9 (1001.5, 1031.6)	1015.0 (990.7, 1041.9)	1022.4 (991.0, 1038.7)	0.97
Cortical porosity (no units)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.069
Mean cortical pore diameter (mm)	0.2 (0.2, 0.2)	0.2 (0.1, 0.2)	0.2 (0.2, 0.2)	0.446
SD of mean cortical pore diameter (mm)	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.766
Periosteal perimeter (mm)	84.9 (77.0, 93.4)	83.3 (75.0, 91.0)	81.8 (75.6, 91.6)	0.795
Endosteal perimeter (mm)	79.9 (73.3, 90.2)	78.6 (70.7, 87.3)	85.5 (70.9, 92.1)	0.716
Stiffness (S in kN/mm)	92.9 (81.1, 116.1)	96.9 (70.8, 114.9)	96.3 (63.5, 109.0)	0.394

	T1DN+	T1DN-	Control	p value
Estimated failure load (F.ult in kN]	4.7 (4.1, 5.8)	4.8 (3.6, 5.8)	4.9 (3.2, 5.6)	0.376
% trab distal load (%)	58.2 (53.8, 63.5)	59.8 (54.9, 63.9)	58.3 (53.8, 66.2)	0.954
% trab proximal load (%)	27.3 (17.9, 32.3)	24.0 (17.9, 30.2)	24.5 (18.5, 29.0)	0.804
Trab average von Mises stress (Tb.VM, in MPa)	5.5 (4.9, 6.0)	5.6 (4.9, 5.8)	5.2 (4.7, 5.7)	0.551
Cort average von Mises stress (C.VM in MPa)	8.0 (7.8, 8.3)	8.1 (7.8, 8.2)	7.8 (7.6, 8.2)	0.246
Cortical area fraction (calculated) (Ct.Ar/Tt.Ar)*100	17.4 (16.2, 20.0)	18.7 (15.5, 23.2)	16.2 (14.1, 21.3)	0.647



*p<0.05 **p<0.017 (p< 0.017 was used as significant for comparison between two groups due to Bonferroni correction for multiple comparisons).

Figure 5-3 Tibia cortical porosity



*p<0.05 **p<0.017 (p< 0.017 was used as significant for comparison between two groups due to Bonferroni correction for multiple comparisons).

Figure 5-4 Tibia connectivity density

14% site

At the 14% site, significant differences were found in several trabecular features both at the tibia (T) and radius (R); trabecular density (tibia T p=0.013 and radius R p=0.19), inner trabecular density (T p=0.01 and R p= 0.007), meta/inner trabecular density (T p=0.01 and R p=0.008), trabecular BV/TV (T p=0.014 and R p=0.021), trabecular number (T p=0.011 and R p=0.007), trabecular separation (T p=0.01 R p=0.005) and trabecular inhomogeneity (T p=0.002 R p= 0.006) (Table 5.4).

Table 5.4 HR-pQCT 14% site results Median (IQR)

	T1DN+	T1DN-	Control	p value
Tibia	n=20	n=20	n=19	
Tibia 14%Total Area (cm2)	558.90 (487.13, 641.68)	522.05 (469.98, 555.25)	543.70 (454.40, 594.70)	0.438
Tibia 14%cortical area (cm2)	176.15 (158.53, 187.85)	180.35 (154.83, 213.28)	186.30 (153.90, 201.90)	0.853

	T1DN+	T1DN-	Control	p value
Tibia 14%trabecular area (cm2)	376.50 (327.88, 449.48)	350.55 (296.08, 392.65)	347.90 (307.40, 392.70)	0.247
Tibia 14%total density (mg HA/cm3)	414.70 (368.65, 464.35)	452.95 (386.85, 480.80)	438.20 (387.30, 454.20)	0.454
Tibia 14%cortical density (mg HA/cm3)	987.15 (957.63, 1002.60)	1001.20 (965.10, 1019.10)	1006.00 (962.30, 1021.50)	0.529
Tibia 14%cortical thickness	1.87 (1.70, 2.16)	2.02 (1.77, 2.39)	2.02 (1.85, 2.20)	0.572
Tibia 14%cortical perimeter	92.70 (85.63, 98.55)	88.00 (85.10, 92.63)	91.00 (83.10, 94.80)	0.359
Tibia 14%trabecular density (mgHA/cm3)	144.85 (100.80, 168.25)	153.20 (114.95, 165.48)	107.30 (101.60, 140.40)	0.042*
Tibia 14%meta trabecular density (mgHA/cm3)	222.80 (188.98, 252.78)	237.10 (192.08, 263.15)	198.90 (184.10, 231.20)	0.158
Tibia 14%inner trabecular density (mgHA/cm3)	90.30 (33.90, 113.53)	84.40 (63.38, 108.10)	55.10 (43.50, 75.70)	0.034*
Tibia 14%Meta/Inn trabecular density (no units)	2.78 (2.21, 4.25)	2.78 (2.10, 3.12)	3.42 (3.11, 4.33)	0.01*
Tibia 14%trabecular BV/TV (no unts)	0.12 (0.08, 0.14)	0.13 (0.10, 0.14)	0.09 (0.09, 0.12)	0.043*
Tibia 14%trabecular number	1.76 (1.27, 1.93)	1.75 (1.44, 2.08)	1.52 (1.36, 1.60)	0.036*
Tibia 14%trabecular thickness	0.08 (0.06, 0.08)	0.07 (0.06, 0.08)	0.07 (0.06, 0.08)	0.482
Tibia 14%trabecular separation	0.49 (0.45, 0.70)	0.51 (0.42, 0.61)	0.60 (0.57, 0.66)	0.032*
Tibia 14%trabecular inhomogeneity	0.23 (0.20, 0.32)	0.22 (0.18, 0.28)	0.29 (0.26, 0.31)	0.008*
Tibia 14%Connectivity Density	0.20 (0.10, 0.37)	0.08 (0.04, 0.24)	0.11 (0.07, 0.23)	0.094
Tibia 14%Cortical tissue mineral density (mg/cm3)	1079.91 (1057.71, 1097.39)	1082.79 (1069.20, 1094.15)	1088.12 (1047.93, 1100.36)	0.819
Tibia 14%Cortical porosity (%)	2.48 (1.2, 3.93)	1.96 (1.42, 2.95)	1.51 (1.1, 2.97)	0.66

	T1DN+	T1DN-	Control	p value
Tibia 14%Mean cortical pore diameter (mm)	0.16 (0.14, 0.17)	0.15 (0.13, 0.17)	0.15 (0.14, 0.16)	0.608
Tibia 14%SD of mean cortical pore diameter (mm)	0.07 (0.05, 0.08)	0.07 (0.05, 0.08)	0.06 (0.06, 0.07)	0.69
Tibia 14%Periosteal perimeter (mm)	89.55 (82.79, 96.51)	84.69 (80.98, 89.02)	86.41 (78.46, 91.28)	0.217
Tibia 14%Endosteal perimeter (mm)	101.03 (82.73, 112.07)	84.81 (78.82, 101.62)	92.36 (77.90, 112.57)	0.237
Tibia 14%Stiffness (S in kN/mm)	233.82 (205.05, 266.32)	245.01 (203.85, 289.66)	242.76 (199.70, 264.41)	0.907
Tibia 14%Estimated failure load (F.ult in kN]	11.70 (10.12, 13.41)	12.10 (10.18, 14.49)	12.10 (9.93, 13.23)	0.912
Tibia 14%% trab distal load (%)	32.05 (20.14, 38.96)	27.31 (25.59, 31.76)	29.07 (22.25, 30.54)	0.521
Tibia 14%% trab proximal load (%)	19.03 (12.60, 24.76)	16.63 (15.10, 18.57)	15.51 (13.08, 19.51)	0.39
Tibia 14%Trab average von Mises stress (Tb.VM, in MPa)	6.15 (5.25, 6.53)	5.99 (5.45, 6.35)	6.01 (5.77, 6.36)	0.499
Tibia 14%Cort average von Mises stress (C.VM in MPa)	9.32 (9.23, 9.38)	9.33 (9.26, 9.37)	9.36 (9.26, 9.41)	0.449
Radius				
	n=13	n=13	n=14	
Radius 14%Total Area (cm2)	534.00 (469.55, 594.45)	526.30 (473.80, 550.65)	535.45 (448.95, 550.23)	0.686
Radius 14% cortical area (cm2)	83.10 (75.70, 91.60)	86.70 (73.80, 97.65)	78.25 (69.80, 91.93)	0.531
Radius 14%trabecular area (cm2)	93.00 (77.65, 107.00)	78.30 (66.30, 105.00)	78.00 (57.55, 93.53)	0.321
Radius 14% total density (mg HA/cm3)	576.90 (539.35, 617.65)	589.00 (562.85, 690.40)	598.85 (541.40, 634.58)	0.463
Radius 14%cortical density (mg HA/cm3)	1042.50 (1027.50, 1080.85)	1061.00 (1039.40, 1072.75)	1057.45 (1031.30, 1073.63)	0.962
Radius 14%cortical thickness	1.56 (1.46, 1.67)	1.65 (1.57, 1.76)	1.57 (1.41, 1.71)	0.451
Radius 14%cortical perimeter	53.00 (50.05, 57.35)	52.00 (46.30, 56.35)	51.40 (45.48, 55.55)	0.559

	T1DN+	T1DN-	Control	p value
Radius 14%trabecular density (mgHA/cm3)	115.40 (88.00, 195.95)	152.40 (111.50, 185.55)	101.50 (74.23, 123.30)	0.03*
Radius 14%meta trabecular density (mgHA/cm3)	207.30 (167.65, 266.80)	221.30 (185.35, 259.00)	191.90 (152.23, 211.30)	0.08
Radius 14%inner trabecular density (mgHA/cm3)	62.80 (31.40, 143.45)	103.50 (59.10, 134.05)	48.55 (19.08, 63.65)	0.013*
Radius 14%Meta/Inn trabecular density (no units)	2.70 (1.84, 4.36)	2.18 (1.88, 3.24)	4.00 (3.17, 9.08)	0.008*
Radius 14%trabecular BV/TV (no units)	0.10 (0.07, 0.16)	0.13 (0.09, 0.15)	0.08 (0.06, 0.10)	0.032*
Radius 14%trabecular number	1.53 (1.08, 1.73)	1.75 (1.49, 1.80)	1.38 (1.03, 1.53)	0.007*
Radius 14%trabecular thickness	0.07 (0.06, 0.09)	0.07 (0.06, 0.09)	0.06 (0.05, 0.08)	0.269
Radius 14%trabecular separation	0.59 (0.50, 0.86)	0.50 (0.47, 0.60)	0.65 (0.58, 0.92)	0.008*
Radius 14%trabecular inhomogeneity	0.25 (0.22, 0.48)	0.23 (0.19, 0.25)	0.30 (0.25, 0.38)	0.009*
Radius 14%Connectivity Density	0.06 (0.04, 0.13)	0.02 (0.01, 0.08)	0.04 (0.02, 0.06)	0.892
Radius 14%Cortical tissue mineral density (mg/cm3)	1121.68 (1111.10, 1142.13)	1121.50 (1107.09, 1137.76)	1124.65 (1093.89, 1137.93)	0.229
Radius 14%Cortical porosity (%)	0.56 (0.39, 1.06)	0.41 (0.26, 1.07)	0.56 (0.21, 1.00)	0.66
Radius 14%Mean cortical pore diameter (mm)	0.15 (0.14, 0.17)	0.14 (0.13, 0.16)	0.14 (0.13, 0.18)	0.377
Radius 14%SD of mean cortical pore diameter (mm)	0.06 (0.05, 0.07)	0.05 (0.04, 0.06)	0.05 (0.05, 0.08)	0.36
Radius 14%Periosteal perimeter (mm)	50.79 (48.22, 55.24)	49.14 (44.35, 53.65)	49.24 (43.74, 56.10)	0.663
Radius 14%Endosteal perimeter (mm)	70.92 (42.94, 85.06)	60.93 (34.31, 81.12)	61.45 (39.74, 74.85)	0.507
Radius 14%Stiffness (S in kN/mm)	94.53 (89.87, 112.01)	100.79 (74.67, 119.40)	96.48 (79.30, 115.78)	0.79
Radius 14%Estimated failure load (F.ult in kN]	4.73 (4.46, 5.61)	4.86 (3.74, 5.93)	4.75 (3.99, 5.80)	0.83

	T1DN+	T1DN-	Control	p value
Radius 14%% trab distal load (%)	15.05 (8.81, 23.67)	11.13 (7.04, 19.16)	9.55 (7.44, 13.39)	0.313
Radius 14%% trab proximal load (%)	8.65 (4.04, 11.30)	5.34 (3.54, 8.85)	5.05 (3.29, 7.90)	0.293
Radius 14%Trab average von Mises stress (Tb.VM, in MPa)	5.50 (4.86, 6.41)	5.39 (4.56, 6.30)	4.77 (4.35, 6.01)	0.345
Radius 14%Cort average von Mises stress (C.VM in MPa)	9.40 (9.31, 9.47)	9.39 (9.27, 9.45)	9.38 (9.26, 9.44)	0.957

The comparison between each pair of groups showed that T1DN- had favorable trabecular microarchitecture with greater trabecular density (**Error! Reference source not found.** A, **Error! Reference source not found.** A), inner trabecular density (**Error! Reference source not found.** B, **Error! Reference source not found.** B) bone volume fraction (**Error! Reference source not found.** C, **Error! Reference source not found.** C) , and trabecular number (**Error! Reference source not found.** E, **Error! Reference source not found.** E) and lower trabecular meta/inner density (**Error! Reference source not found.** D, **Error! Reference source not found.** D), trabecular separation (**Error! Reference source not found.** F, **Error! Reference source not found.** F), and inhomogeneity (**Error! Reference source not found.** G,**Error! Reference source not found.** G), both at the tibia and radius (Table 5.5). For most of these features, there was a trend to the T1DN+ group to follow the same pattern, but besides trabecular inhomogeneity it did not reach statistical significance (Table 5.5, **Error! Reference source not found.**, **Error! Reference source not found.**). There were no differences in bone geometry, cortical features or bone strength measured by finite element analysis. (Table 5.4

Table 5.5 Percentage differences in trabecular features between T1DN- and T1DN+ and control

	T1DN- vs control (%)	p value T1DN- vs control	T1DN+ vs control (%)	p value T1DN+ vs control
Tibial trabecular density (mgHA/cm3)	↑43	0.003**	↑35	0.046*
Tibial inner trabecular density (mgHA/cm3)	↑53	0.002**	↑64	0.034*
Tibial Meta/Inn trabecular density (no units)	↓19	0.002**	↓19	0.044*

Tibial trabecular BV/TV (no units)	↑43	0.003**	↑36	0.047*
Tibial trabecular number	↑15	0.005**	↑15	0.023*
Tibial trabecular separation	↓15	0.005**	↓18	0.021*
Tibial trabecular inhomogeneity	↓25	0.001**	↓22	0.008**
Radius trabecular density (mgHA/cm3)	↑50	0.006**	↑14	0.057
Radius inner trabecular density (mgHA/cm3)	↑113	0.002**	↑29	0.031*
Radius Meta/Inn trabecular density (no units)	↓45	0.002**	↓32	0.035*
Radius trabecular BV/TV (no units)	↑50	0.006**	↑14	0.064
Radius trabecular number	↑27	0.002**	↑11	0.072
Radius trabecular separation	↓23	0.001**	↓9	0.076
Radius trabecular inhomogeneity	↓24	0.001**	↓17	0.118

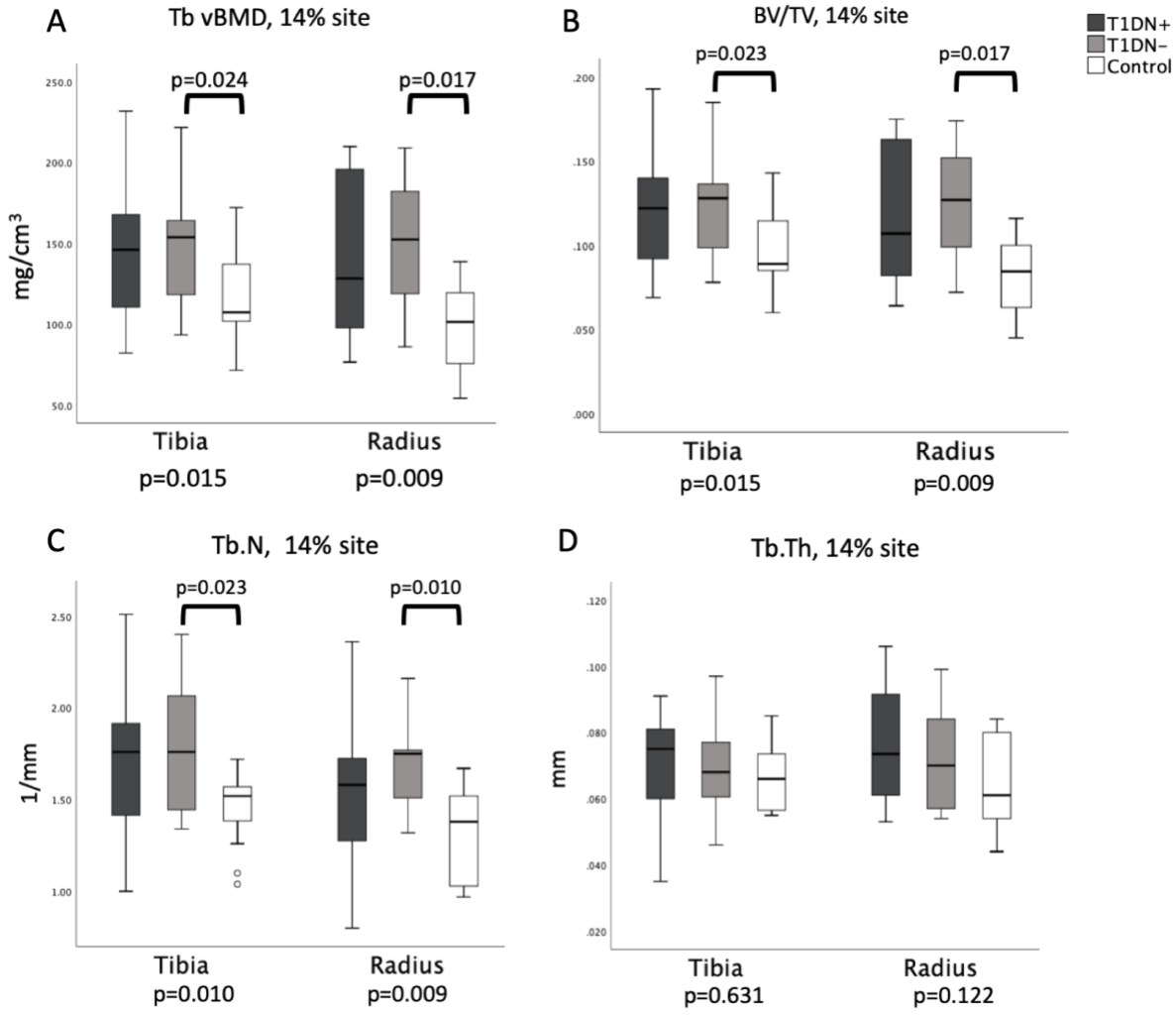


Figure 5-55-6 Tibia and Radius 14% site

A Tb.vBMD tibia and radius; B BV/TV tibia and radius; C Tb.N tibia and radius D Tb.TH tibia and radius

BTM results

PINP and CTX were decreased in participants with diabetes (T1DN+ and T1DN-) compared to controls. PINP was 34% (p=0.006) and 28% (NS) lower in T1DN- and T1DN+ compared to controls, while CTX was 87% (p=0.016) and 90% (p=0.011) lower, respectively. No difference was found between diabetic groups (T1DN+ and T1DN-).

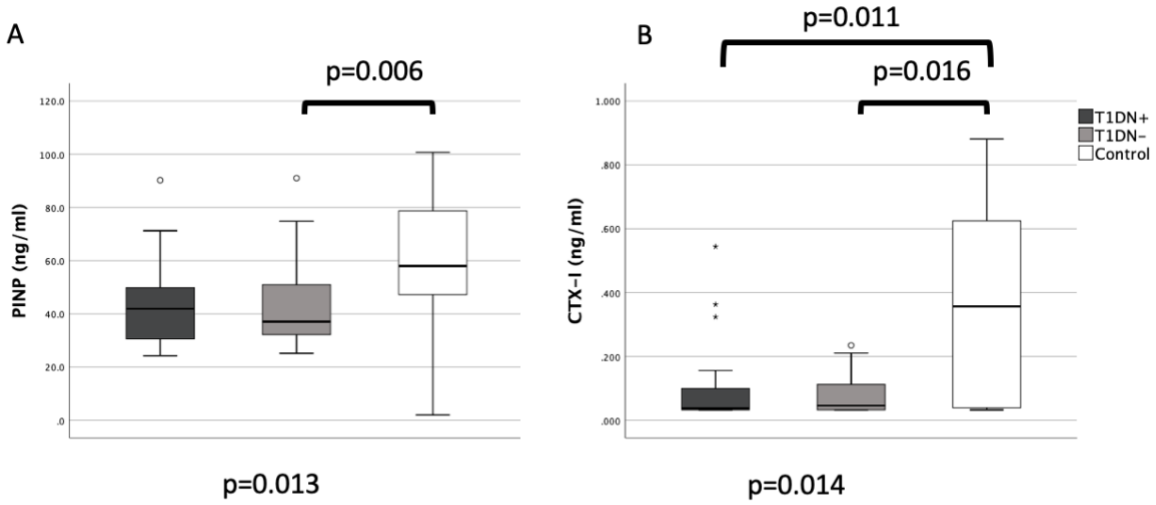


Figure 5-7 PINP (A) and CTX (B) in the three groups

Discussion

Summary of the main findings

There was no difference in aBMD measured by DXA in the three groups. In the HR-pQCT assessment, trabecular microarchitecture was preserved in T1DN- compared to controls at distal limbs. The pattern was more obvious at the 14% site, where we report robust findings of favourable trabecular microarchitecture both at the radius and the tibia in T1DN- in a consistent pattern. There was a trend for the same pattern in T1DN+. At the standard site (more distal)

microarchitecture was not different between T1DN- and controls in any of the features assessed. When we assessed the influence of neuropathy, T1DN+ had higher cortical porosity compared to T1DN- and also significantly higher connectivity compared to T1DN- and controls. BTM were decreased in participants with diabetes (T1DN+ and T1DN-) compared to controls and no difference was found between the diabetic groups (T1DN+ and T1DN-).

aBMD

We found no differences in the measurements made by DXA between the groups. Data in aDXA in diabetes are conflicting but overall a decrease in aBMD is observed in T1D (17, 84). In this study, the main outcome was measured by HR-pQCT. For HR-pQCT measurements gender, height and weight could affect the results and we chose to control these features by matching the participants. These features can also affect BMD. Although the matching was not fully successful there were no significant differences in these features between the groups. We speculate that the attempt to match might have influenced potential differences between the groups. For example, body weight is an important determinant of BMD. Therefore, the matching for this characteristic might have affected the aBMD results.

Furthermore, other features could also influence the aBMD. There is evidence that poor metabolic control has a negative impact on aBMD in growing children (171). Therefore, a T1D onset before peak bone mass accrual and poor metabolic control during the growing period might have a marked negative impact on aBMD. In this study, in 17 (7 T1DN-/10 T1DN+) participants, T1D onset was earlier than 18 years-old. We did not have access to the metabolic control during growing period and we could not assess a potential impact of metabolic control in early onset T1D in aBMD. Additionally, the decrease in aBMD reported in T1D is not substantial. In a meta-analysis, Vestergaard described a decrease in BMD Z-score at spine (mean ±SEM -0.22±0.01) and hip (-0.37±0.16) (84). Therefore, it is possible that our sample size was not big enough to detect this difference.

HR-pQCT findings

Trabecular compartment

14% site findings

A number of significant differences in trabecular features were found in the 14% site. Greater BV/TV, trabecular density, inner trabecular density and trabecular number and lower trabecular separation and inhomogeneity were consistent findings at the radius and tibia when comparing T1DN- and controls and a trend when comparing T1DN+ and controls. Trabecular meta density refers to trabecular density of the outer 60% of the trabecular region, while trabecular inner density refers to density of inner 40% of trabecular region. The trabecular meta-inner density is the ratio between the two measurements. A decreased meta/inner density showed that the trabecula in the outer region are less preserved than in the inner region. Noteworthily, meta density was not different between the groups (T p=0.158; R p=0.08).

Standard site findings

At the standard site, no difference was found while comparing T1DN- and controls, but the T1DN+ group showed higher trabecular connectivity when compared to controls and T1DN. Generically, connectivity is a measure of the degree to which a structure is connected multitudinously to other structures (203). When described as in index, the index is derived from the Euler number (203). In bone microarchitecture, connectivity characterises the multiplex of trabecular connections (70). The index considers the number of objects, the number of cavities fully surrounded by bone and the number of connections that must be broken to separate the structure in two parts. Connectivity depends on structure size and it is normalised by dividing by the total volume resulting in connectivity density (Conn.D) (70). Connectivity does not consider the thickness of trabeculae and does not make a distinction between plates and rods. Consequently, connectivity is a measure of the degree of multiple connections, no matter the shape of the connections (203). Higher connectivity is often a favourable finding, however, if one plate is transformed in several rods connectivity will increase.

Despite being part of the standard bone microstructure analysis, connectivity is not one of the minimal set of variables reported for trabecular analysis and might not be reported unless specifically discussed (70). For example, Macdonald et al in the cross-sectional study that evaluated age-related changes in bone microarchitecture did not report connectivity (204).

BTM

PINP was decreased in T1DN- compared to controls and CTX was decreased in T1DN- and T1DN+ compared to controls. No difference was found between the diabetic groups (T1DN+ and T1DN-). These findings suggested that bone turnover is low in T1D, regardless of neuropathy. These findings agree with previous literature. Lower levels of CTX have been described in both T1D and T2D in individual studies and in a meta-analysis that summarised BTM in diabetes (25, 28, 42). Lower levels of PINP have also been reported in T1D and T2D, but PINP was not assessed by the meta-analysis due to lack of sufficient data (25, 28, 42). The mechanisms involved in this decrease in the bone turnover are no fully understood but evidence suggest that this might be associated with hyperglycemia directly and indirectly. Some evidence suggest that hyperglycemia has a direct inhibitory effect on bone cells (205) In addition, hyperglycemia favors the formation of AGEs and there is also evidence that AGEs have a negative impact on bone turnover (206, 207), as discussed below (mechanisms).

Mechanism

Hyperglycemia 1- direct effect

The trabecular findings might be associated with the low bone turnover. A number of studies have described low bone turnover associated with diabetes (25, 28, 208) and this might be linked with hyperglycemia. There is evidence for a direct and indirect effect of hyperglycaemia in the skeleton. In vitro studies have shown that chronic hyperglycaemia inhibits osteoclast (209) and osteoblast differentiation and activity (205). A recent study compared histomorphometry from premenopausal T2D women with good (n= 10, HbA1c<7%) and poor metabolic control (n=16, HbA1c>7%) to age and race matched controls without diabetes. The group reported greater

BV/TV in T2D with good control compared to the non-diabetic control group and borderline findings in the poor control group (p=0.05). Furthermore, there was greater TB.N and lower trabecular separation in both T2D groups compared to non-diabetics regardless of the metabolic control. Interestingly, only the poor metabolic control group showed reduced osteoid thickness compared to controls without diabetes. There was a negative correlation between HbA1c levels and parameters of osteoid production, such as osteoid thickness and osteoid surface (40). These findings suggested preservation of trabecular structure in T2D and a reduction in bone formation associated with hyperglycaemia.

Hyperglycemia 2- indirect effect via AGEs

As previously discussed, glucose can bind to protein residues, which results in the formation of AGEs. The process is exacerbated by hyperglycaemia, which favours the formation and accumulation of AGEs, especially in long-lived tissue proteins such as collagen. AGEs might have a central role in the decrease in bone turnover in diabetes, especially at the trabecular compartment.

In vitro studies, based on histology and in the release of products of collagen degradation have shown that AGEs decrease osteoclast and osteoblast activity (206, 207). In addition, osteoclastogenesis was also strongly inhibited by AGEs (206). Both osteoclast and osteoclast progenitors express AGEs receptors. This evidence suggests that AGEs could decrease osteoclastic differentiation, osteoclast-induced bone resorption and also osteoblast activity (206).

AGEs are not exclusive of diabetes, since AGEs formation is associated with ageing. In individuals without diabetes, Viguet-Carrin et al assessed the association between collagen cross-links and trabecular microarchitecture properties of human vertebral bone. There was a positive correlation between pentosidine content and trabecular number and connectivity density and a negative correlation between pentosidine and trabecular separation. These findings were independent of trabecular bone volume, suggesting that pentosidine was associated with preserved trabecula. Pentosidine was associated with denser and rod-like, rather than plate-like trabecular network. This finding suggested that the preserved trabeculae contains mostly aged

bone that has undergone little remodeling and thus contains higher amounts of AGE cross-links such as pentosidine (16).

In vitro studies have suggested that AGEs inhibit bone turnover (206, 207). In a low bone turnover states, AGEs would not be cleared, resulting in the accumulation of AGEs. Therefore, the mechanism seems to be perpetuated. However, it is not clear if the accumulation of AGEs led a decrease in bone turnover or if the low bone turnover led to the accumulation of AGEs.

AGEs formation is increased by the hyperglycemia associated with diabetes. In a small cross-sectional case-control study of transiliac bone biopsies from subjects with T1D with a fragility fracture (T1DFx+, n=5) compared to healthy age- and sex-matched non-diabetic controls (n=5) and T1D without a fracture (T1DFx-, n=5), pentosidine content in trabecular bone was higher in T1DFx+ compared to controls (37). This finding agrees with *in vitro* evidence that suggested that trabecular bone is more susceptible to the formation and accumulation of glycation products than cortical bone (45). The T1DFx+ group had less surface covered with osteoblasts and osteoid and lower mineralizing surface, reflecting a lower bone formation rate and activation frequency. The degree of mineralization was higher in T1DFx+ compared both to controls and T1DFx (37), also reflecting lower bone turnover. Furthermore, there was a positive correlation between bone pentosidine content and HbA1C. These findings suggested that AGEs content was associated with hyperglycaemia, lower bone turnover and fractures in T1D.

Taken together, this evidence suggests that AGE formation and deposition in bone might have an important role in the low bone turnover observed in people with diabetes. This low bone turnover could result in preservation of trabecular microarchitecture observed at the 14% site. Interestingly, there was a trend to the same pattern of microarchitecture in T1DN+. Since T1DN+ is considered a group with more advanced diabetes disease, as it is affected by neuropathy, a microvascular complication, we speculate that it is possible that other mechanisms might have affected this population.

Impact of trabecular findings in bone strength and fracture risk

The trabecular compartment has an important role in bone strength. A case-control study assessed postmenopausal women with and without fractures using HR-pQCT and reported lower

trabecular density, number and thickness and higher trabecular separation and inhomogeneity in women with fractures (210). Data from the OFELY study, that prospectively assessed the association of bone microarchitecture (HR-pQCT) and the risk of fractures reported that each quartile decrease in radius Tb.N was associated with an increase in the risk of fractures (HR 1.32 95%CI 1.08,1.61). Conversely, the decrease in radius Tb.S was associated with a decrease in the risk of fractures (HR 0.76 95%CI 0.63-0.92) (211). Therefore, the trabecular findings in 14% site are favourable findings and would be associated with a decrease in the risk of fractures

In prospective studies that evaluated the bone microarchitecture as predictor of fracture risk in postmenopausal women, each quartile decrease in Conn.D was associated with an increase in the risk of fractures with an HR 1.49 (1.05–1.30), (adjusted HR for age, current smoking, falls in the past year, prior fracture, use of osteoporosis-related drugs, and total hip BMD) (211).This data suggest that connectivity is a favourable finding.

Cortical compartment findings

At the standard site, cortical porosity was higher in T1DN+ than T1DN-. Cortical pores are images of the spaces occupied by vascular structures in the cortical bone. Cortical bone is a compact structure formed by osteons. Osteons are the fundamental bone unit, cylindric structures formed by a central harversian canal where vascular capillaries and nerves are located, surrounded by layers of bone cells and mineralised bone matrix. These structures connect to each other by Volkmann's canals. In cross-section, Harversian and Volkmann's canals appear as a void volume, resembling pores. Human cortical bone displays a multiscale net of pores, which form a three-dimensional network of interconnected canals with metabolic function and impact on bone biomechanics. This structure is not static as it is susceptible to remodelling. In bone morphologic analyses, this structure is visualised as pores, namely cortical porosity.

The increase in cortical porosity was fond in T1DN+, the group with neuropathy. Neuropathy is a diabetic microvascular complication. It is characterized by abnormal nerve function caused by vascular and metabolic abnormalities. Neuropathy could potentially have an impact on the skeleton through two mechanisms; a direct impact of nerve damage or the same microvascular mechanism that affects the nerves could also affect the skeleton.

Mechanisms 1 Vascularization

The vasculature has a key role in skeletal development, growth and maintenance (212). In animal models, Kusumbe et al investigated the link between angiogenesis and osteogenesis (213). The group reported that a specific kind of vessels mediates the growth of bone vasculature, maintains perivascular osteoprogenitors and couples angiogenesis to osteogenesis, highlighting the importance of vascularization for bone turnover. The group also reported that the abundance of these vessels is reduced with ageing. Transendothelial migration of cells from both the osteoclastic and osteoblastic lineage is likely to be tightly regulated by the endothelium (214) . These findings suggest that vascularization plays a key role in bone remodelling. Consequently, abnormalities in bone blood supply could impact in bone homeostasis.

Low bone turnover might also have an impact on cortical porosity. In histomorphometric studies of people without diabetes, Andreasen et al assessed iliac bone specimens of 35 women from 16 to 78 years old and found an increase in cortical porosity with ageing (215). This increase was mainly a result of an increase in pore size. The authors claim that in the remodelling process in existing pores, there is a delay or absence on the formation phase, resulting in large and coalescing pores and increase in cortical porosity (215). Diabetes is characterised by low bone turnover. Hence, it is possible that this delay or absence on the formation phase could be exacerbated, resulting in increased cortical porosity.

Mechanisms 2 Innervation

Furthermore, some evidence suggests that innervation might also play a role in bone metabolism. There is evidence for a neuronal control of bone remodelling, mediated mainly by the beta-adrenergic system (51). It is also known that vascular and nerve components occupy the harversian canals but the role of each of these structures in bone remodelling is not characterised. Recent histomorphometric studies have investigated the nerve distribution in iliac crest of patients with primary hyperparathyroidism. In the cortical compartment, nerves were only visualised in the cortical pores, not in the cortical bone matrix. Moreover, the density of innervation was 5-fold higher in the cortical bone compared to the periosteum and bone marrow.

In the bone marrow, more than 90% of the nerves were associated with vascular structures and innervation was denser above remodelling units. The authors claim that this anatomical link between innervation and bone remodelling suggested a role of innervation in the bone remodelling process (216). This evidence came from iliac bone in a subset of patients with high bone turnover and whether this would apply to long bones or individuals with normal or low bone turnover is unknown. However, if nerves are involved in bone remodelling, this process could be disrupted in patients with neuropathy. Therefore, there is evidence that both vasculopathy and neuropathy could have a negative impact on the bone remodelling process. As both vascularization and innervation might be impaired in diabetic neuropathy, we speculate that this could be associated with the increase in cortical porosity observed.

Impact of cortical findings in bone strength and fracture risk

An increase in cortical porosity is an unfavourable finding. Morphologic analyses by quantitative computed tomography have shown an increase in cortical porosity with ageing (204). A cross-sectional study by Macdonald et al evaluated age-related changes in bone microarchitecture and strength at the distal radius and distal tibia in 644 adults aged 20 to 99 years. The study reported changes in bone total area, trabecular compartment (trabecular number, trabecular thickness) and an increase in cortical porosity both in men and women with ageing. Finite element analysis suggested that these findings were associated with a decrease in bone strength. Several other studies have reported an association between cortical porosity and bone fragility (26, 217, 218). *Ex- vivo* analyses suggest that an increase in porosity increased crack propagation thorough bone and decreased the peak stress that can be tolerated before a fracture. The ability of bone to deform without cracking also decrease when porosity increases (217). Therefore, an increase in cortical porosity would be associated with an increase in the risk of fractures and could contribute to bone fragility in diabetes.

HR-pQCT in diabetes – previous literature standard site

Conflicting results have been reported in microarchitecture in diabetes with favourable findings mainly in T2D and neutral and unfavourable findings both in T1D and T2D. Findings were reported both at cortical and trabecular compartments. However, there is an important variability in the study design. Some studies are cohort studies that compared T2D and non-T2D in the population (27, 31, 32). Others recruited participants with diabetes and age and height matched healthy controls (25, 26). Two studies, one in T1D and other in T2D stratified the analysis according to the presence of microvascular complications while the others did not take this into account. Some studies compare participants with diabetes with controls while others compared T2D participants with and without fractures.

The results at the standard site agree with previous literature that reported no difference in microarchitecture when comparing people with T1D MVD- and controls (25). Similar results were also reported for T2D (28). Our results also agree with previous data that reported an increase in cortical porosity in diabetes. Previous results reported this finding in T2D regardless of MVD (26, 27, 32) or in the group with MVD+ (28), while we report the finding in T1DN+. Shanbhogue et al, the previous study in T1D, reported a 25% decrease in the cortical area but no abnormalities in cortical porosity.

Shanbhogue et al, in the T1D study, also reported a decrease in vBMD both in the radius and the tibia, while comparing T1D MVD+ with non-diabetic controls. We did not find differences in volumetric BMD in our study, however, our sample was 30% smaller than the previous study. While comparing T1D MVD+ and T1D MVD-, Shanbhogue reported several unfavourable findings both at the trabecular and cortical compartment and reduced bone strength at both sites. These findings are different from our findings when comparing T1DN+ and T1DN-. However, the study design is different; while we selected DSPN as the MVD of choice and performed a careful assessment to categorise participants into the T1DN+ and T1DN- groups, Shanbhogue used vibration threshold test, microfilament test, and examination of foot reflexes to establish neuropathy diagnosis. This clinical test isolated is much less accurate for the neuropathy diagnosis. None of the T1DN- participants had nephropathy, but some of them have reported previous retinopathy. In addition, we aimed to match the groups, so we recruited the same

number of males and females in each group while the groups were unbalanced in Shanbhogue. Finally, Shanbhogue sample size was also bigger (30% for T1DN+ group and 45% for T1DN-) (25).

HR-pQCT in diabetes – previous literature 14% site

There is only one previous study reporting the 14% site in diabetes, in T2D. Nilsson et al reported greater cortical area and failure load at both the tibia and radius and lower cortical porosity at the radius while evaluating a cohort of 75-80 years old women, 954 controls and 99 with T2D. This study assessed elderly women with T2D, a quarter of them with recently diagnosed T2D and the T2D group had greater BMI than controls. Although statistical adjustments for age and BMI were applied, this should be considered while interpreting the results. As in our study, favourable results were reported in the group with diabetes. However, while we report favourable findings in the trabecular compartment, Nilsson et al reported findings in the cortical compartment (31). Nevertheless, the great diversity in the population and study design between the two studies prevents further comparations.

HR-pQCT sites inconsistencies

We reported inconsistent findings at the 14% and standard sites, with several favourable findings at the 14% site in T1DN- and a trend in T1DN+ and only an increase in connectivity at the standard site.

These differences might be associated with the proportion of trabecular bone at the sites measured. Schlenker and Vonseggen (219) have investigated the distribution of cortical and trabecular bone mass along the radius length. At distal radius approximately at the area measured by HR-pQCT the percentage of trabecular bone varies from 50 to 85% with an important variation within a small distance. For example, at 1.61 cm from radial styloid tip the percentage of trabecular (Tb) and cortical (Co) bone were (Tb 76.7 and Co 23.3%) while at 2.09 cm the percentages were (Tb 46.8 and 53.2%) (Table 5.6). The standard site was measured at the fixed distance of 9.5/22.5 mm from the reference line placed at the notch on the articular surface, following standard procedures. Conversely, for the 14% site, the limb length was measured and the measurement site placed at the 14% length. We speculate that this might have resulted in a

better consistency in the site measured between the participants and have reduced potential noise in the measurements due to positioning variations. Although there was no difference in participants' height, Schlenker and Vonseggen data on the radius suggested that even small differences in the measuring site could result in important variations in the trabecular content between the two sites.

Table 5.6Percentages of trabecular and cortical bone in the radius of a 43-year-old woman according to Schlenker and Vonseggen

Radius length	Trabecular bone (%)	Cortical bone (%)
0.62 cm	68.0	32.0
1.11 cm	85.5	14.5
1.61 cm	76.7	23.2
2.09 cm	46.8	53.2
2.60 cm	19.1	80.9
3.09 cm	9.3	90.7
4.93 cm	1.2	98.8
8.70 cm	3.9	96.1

We found an increase in cortical porosity in T1DN+ at the tibia at the standard analysis, but not at the 14% site. Neuropathy is a length dependent damage process and we speculate if the finding only at the most distal site could reflect the neuropathy distribution pattern.

Finally, the increase in cortical porosity was found only at the tibia. There are two potential reasons for this finding. Firstly, due to movement artefacts, 9 radius scans could not be included in the radius analysis, reducing the sample size. Additionally, neuropathy affects mainly lower limbs with only advanced disease affecting the upper limbs. Consequently, if neuropathy is a determinant of the increase in cortical porosity, it would be less likely to be detected at the radius than at the tibia.

Limitations

This study has limitations We aimed to match the participants in the three groups for several variables. For example, height could have an impact in the site measured. In addition, age and weight/BMI could affect microarchitecture and act as confounders. However, recruitment was a

challenge and participants were not fully individually matched. Although the individual match was not possible, the groups were similar in regards to these features.

Furthermore, neuropathy is a disease with a broad spectrum. We used DSPN defined by clinical symptoms and an objective measure of nerve conduction as the main feature to characterise the participants as defined by the Toronto Consensus (6). This is mainly an assessment of large fibre damage. It is possible that other assessments would result in different categorization.

Additionally, some patients in the non-neuropathy group had a negative assessment for neuropathy but had a previous history of retinopathy (8 background retinopathy, 2 proliferative retinopathy). Therefore, the T1DN- group is not the same categorization as MVD-.

Although HR-pQCT is the *in vivo* scanner with the highest resolution available for human bone measures the resolution is limited to the 82 μm voxel size. This is close to the human trabecular dimension (71). In addition, only pores greater than 82 μm would be detected and over 60% of cortical pores are less than 100 μm in diameter (217). Therefore, only the minority of greater pores would be detected in the cortical porosity analysis. Finally, we assessed participants at the 14% length of the radius and the tibia an unusual site for scanning and we used the same protocol used in the standard analysis to analyse these images. The standard protocol has been validated against the gold standard method high-resolution micro-computed tomography, using cadaveric samples (220). The protocol has not been validated to this site.

Conclusion

There is an impact of T1D in bone microstructure and this impact is influenced by neuropathy.

Diabetes is associated with lower bone turnover (associated with hyperglycaemia and AGEs) and this might have a paramount role on bone findings. We speculate that the low bone turnover would preserve trabecular microarchitecture resulting in a favourable microarchitecture pattern which was seen in T1DN- at the 14% site. Variations of the site measured might have prevented the detection of this findings at the standard site. This mechanism could also explain the aBMD preservation. With the disease progression and the development of neuropathy other mechanism associated with nerve functioning abnormalities and microvascular blood flow might also affect bone remodelling. These mechanisms could explain the increase in cortical porosity

observed in T1DN+. There is strong evidence for a crucial role of the vasculature in bone remodelling. However, nerves and vessels are components of the harversian canals and it is possible that the nerves might also be involved in the process.

Chapter 6

Thesis discussion

Chapter 6 Discussion

Introduction

Previous literature suggested an increase in the risk of fractures in diabetes. The risk seemed to be higher in T1D than in T2D but the effect of several features such as fracture site, gender, age, BMI and diabetes-related features such as diabetes duration, insulin use and the presence of complications has not been fully explored. This thesis investigated the risk of fractures in diabetes. The first meta-analysis (chapter 3) investigated the risk of hip and non-vertebral fractures in diabetes and how this risk was affected by several features associated with the patients and the disease. The second meta-analysis (chapter 4) investigated the risk of peripheral fractures in diabetes (wrist and ankle), the sites assessed by HR-pQCT. Finally, a clinical study was conducted to assess peripheral microarchitecture in patients with T1D with and without neuropathy. This study investigated the effect of diabetes and also neuropathy, one of its main complications on bone density and peripheral microarchitecture(chapter 5). Our hypothesis was that the increased in the risk of fractures in diabetes would be associated with impaired microarchitecture.

Chapter three results summary

A systematic review and meta-analysis on the risk of hip and non-vertebral fractures in diabetes was conducted. We also investigated whether this risk was affected by age, BMI, diabetes duration, insulin use and the presence of complications. We found a significant increase in the risk of fracture in diabetes both for hip (RR 1.52, 95% CI 1.42-1.63) and for non-vertebral fracture (RR 1.20, 95%CI 1.14-1.27). The increase in the risk was greater for insulin users and longer diabetes duration, at both sites. At the hip, the risk was higher in the younger population, women, and those with T1D. Some evidence suggests that poor metabolic control and diabetic complications could affect the risk of fractures, but there was not enough data to investigate the effect of these features on the risk of fractures.

Chapter four results summary

In the second meta-analysis, the risk of peripheral fractures in diabetes was investigated. There was a discordant pattern at the wrist and ankle. While at the wrist the risk was decreased (RR 0.85 95% CI 0.77 – 0.95), at the ankle the risk was increased (RR 1.30 95%CI 1.15 – 1.48). The sample included mainly T2D participants and the pattern was similar to the risk pattern observed in obesity. As obesity is highly prevalent in T2D we speculated that the risk observed in diabetes were mainly driven by this feature. Recently, our group investigated bone density and microarchitecture in obesity and found greater aBMD and favourable microarchitecture in obesity. There is conflicting data in microarchitecture in T2D and few data in T1D. However, it would be interesting to investigate bone microarchitecture at these sites.

Chapter five results summary

Bone microarchitecture in T1D was also investigated. As the risk of fractures was higher in T1D I speculated that potential mechanisms associated with the increased risk of fractures in diabetes would be more evident in this population. Some evidence in literature also suggested a role for microvascular complications. Therefore, the study was designed to investigate the effect of T1D and also one of the microvascular complications. Neuropathy was the microvascular complication selected. Participants with T1D with and without neuropathy were recruited and also healthy controls without diabetes. Areal BMD and HR-pQCT were assessed. BMD was assessed at lumbar spine and hip and no difference was detected between the groups. HR-pQCT was assessed at the radius and tibia. We measured the standard site (ultra-distal) and also a less distal site, at 14% of the length of the radius and tibia. The ultra-distal site is rich in trabecular bone and the evaluation of the cortical compartment could be limited. Furthermore, previous studies have reported abnormalities in cortical porosity. Therefore, in order to investigate the cortical compartment more extensively a less distal site was also evaluated. Surprisingly, the 14% site showed preserved trabecular structure and no abnormalities in the cortical compartment in the diabetic groups, in particular in the T1DN- group. At the standard site, cortical porosity was increased in the group with diabetes and neuropathy at the tibia. However, there were no differences in bone strength estimated by finite element analysis. Since bone turnover markers

are decreased in diabetes, bone turnover is suppressed. I speculated that the bone turnover suppression could prevent bone loss and preserve trabecular microarchitecture. Conversely, cortical porosity was increased only at the tibia in the group with neuropathy. This finding suggested that vascular and/or neural integrity might also be important to bone remodelling and consequently, bone microarchitecture.

Bone turnover is decreased in diabetes and this is expected to preserved trabecular structure. However, initially, low bone turnover seems not to be associated with cortical porosity. Preserved bone microarchitecture was more evident in T1DN- (a trend was found in T1DN+ but it did not reach statistical significance). Conversely, increased cortical porosity was found only in T1DN+, suggesting that features specific to this group might be involved. Neuropathy is associated with abnormal vascular and nerve function (7). It is possible that these abnormalities could compromise bone remodelling and influence cortical porosity. Neuropathy can be associated with abnormal muscular function and this could also affect bone structure (6). In addition, it is important to consider the limitations of the method; currently, HR-pQCT is able to detect only pores greater than 100 μm and the majority of the pores are smaller than this threshold. Cortical porosity increases with age (204). Since the average age of the population in this study was around 48 years it is possible that a subtle variation in cortical porosity would not be detected, as we could only detect big pores. Therefore, an increase or decrease in small pores, smaller than this threshold would not be detected. Nilsson et al has previously assessed the cortical compartment at the 14% site in an elder cohort of participants with T2D, 1/4 of them with a recent diagnosis. Interestingly, they reported a decrease in cortical porosity in the diabetic population (31). It is possible that in this elder population with greater pores at baseline, the decrease in bone turnover would be associated with the decrease in cortical porosity reported. However, this was an unexpected finding, as the decrease in cortical porosity would increase bone strength. In addition, an increase in cortical porosity in the diabetic group with microvascular disease has been reported both in T1D (25) and in T2D (28). These findings suggest that cortical porosity is affected in diabetes and that microarchitecture is not an important determinant of bone fragility in this population.

Peripheral fracture meta-analysis and radius HR-pQCT results

The microarchitecture study results agreed with the peripheral meta-analysis results. At the radius, the meta-analysis showed a 15% decrease in the risk of fractures in diabetes. Previous studies showed that wrist fractures are associated with bone loss and lower BMD (221). Furthermore, previous studies also reported an increase in BMD and favourable microarchitecture in obesity (67). Consequently, a decrease in the risk of wrist fractures would be expected in the obese population. Obesity is highly prevalent in T2D and the studies included in the meta-analysis reported data mainly from T2D. Therefore, the increase in the risk of fractures in diabetes was initially associated with obesity. However, in the T1D clinical study, only participants with T1D were included and there was no significant difference on weight or BMI between the groups. Therefore, no influence of weight/obesity would be expected. The analysis showed favourable microarchitecture at the radius at the 14% site. Even though there was no difference in bone strength estimated by finite element analysis, favourable microarchitecture would be expected in a site where there is a decrease in the risk of fractures. The radius fracture meta-analysis result agreed with the microarchitecture pattern found in the participants with T1D, especially the group without neuropathy.

Peripheral fractures meta-analysis and tibia HR-pQCT results

Conversely, ankle fractures are not considered typical osteoporotic fractures (196)(197). Ankle fractures are not associated with lower axial BMD and do not predict future fractures. However, unfavourable microarchitecture has been described in people with ankle fractures (197). Furthermore, ankle fracture was associated with obesity (196). The meta-analysis on the risk of ankle fractures in diabetes showed a 30% increase in the risk. Initially, this increased risk was associated with obesity, highly prevalent in T2D, the main population included in the meta-analysis.

In the clinical study in T1D, favourable trabecular microarchitecture was found at the 14% site and an increase in cortical porosity was found at the standard site. While the increase in cortical porosity could contribute to an increase in the risk of ankle fractures, the favourable trabecular pattern seemed contradictory.

Previously, a case-control study that compared 31 postmenopausal women with and without ankle fractures reported no difference in spine BMD, total or regional ankle BMD and calcaneal broadband ultrasound attenuation between the two groups (196). The ankle fractures group were 10 kg heavier than the non-fractures group. These findings suggested that ankle fractures are not associated with axial or local BMD. In addition, a recent consortium of several microarchitecture cohorts reported that ankle fractures were not associated with any measure of BMD or HR-pQCT (222). Finally, Evans et al has shown favourable microarchitecture in an obese population, despite a higher risk of ankle fractures (67). Therefore, ankle fracture risk seems not to be associated with microarchitectural features at the tibia. Therefore, the microarchitectural findings in the clinical study seem not to influence the risk of fractures at this site. We speculate that the increased risk of fractures found in the meta-analysis is associated with obesity in the T2D population. The increase in the risk of ankle fractures is due to an increase biomechanical forces applied to the limb during falls.

Research hypothesis

The results showed an increase in the risk of hip, non-vertebral and ankle fractures in diabetes. The initial hypothesis was that impaired microarchitecture could be one of the mechanisms associated.

An increase in cortical porosity was found at the standard site at the tibia. However, this is an isolated finding, at a site where the risk of fractures is not directly associated with bone density or microarchitecture. In addition, in the trabecular compartment, the microarchitecture was favourable. Therefore, it is unlikely that unfavourable microarchitecture is an important mechanism associated with bone fragility in diabetes.

Speculations

There is a decrease in bone turnover in diabetes (25). Although this could result in preserved bone mass this could also be associated with a decrease in microdamage repair. The accumulation of microdamage could result in an increased risk of fractures.

Several studies on bone microarchitecture in diabetes have reported conflicting results. Neither bone quantity, assessed by DXA, nor bone quality, assessed by HR-pQCT, could explain the bone fragility in diabetes. The next step would be to assess bone material properties. Two studies have previously assessed BMSi using reference point indentation (31, 34). Both of them have reported a decrease in BMSi in T2D. Some evidence suggested that BMSi could correlate to bone toughness and it is possible that it could capture the abnormalities in bone in diabetes (223).

We speculate that the hyperglycemia and AGEs deposition are associated with a reduced bone turnover. As bone turnover is low, bone damage repair would also be affected. This could be associated with bone fragility in diabetes. This is a speculative theory but might be supported by some studies in atypical femur fractures.

Atypical femur fractures (AFF) are associated with decreased microdamage repair (224). Previous studies suggested that the risk of AFF is increased in diabetes (225) . Data from the SOF study has shown that diabetes was associated with an increase in the relative hazard (RH) for AFF (RH: 2.97, (1.47, 6.00) p= 0.005). Age (RH: 2.04 per 5 years, (1.59, 2.63) $p < 0.001$) and femoral neck BMD (RH/SD decrease: 1.41, (1.01, 1.96) p= 0.04) were the other significant risk factors. While bisphosphonates use is known to be associated with an increase in the risk of AFF, in the SOF analysis the association was not significant with a RH of 2.40 (0.97, 5.95). However, the number of AFF was low (159).

These findings raise the concern about the use of anti-resorptive drugs in this population. Previous studies have shown similar efficacy of anti-resorptive drugs in diabetes. However, the pathophysiology of bone fragility in diabetes is not established. As the baseline bone turnover is suppressed, additional suppression may prevent microdamage repair. If the low bone turnover is the main mechanism driving bone fragility in diabetes concerns could be risen over the safety of anti-resorptive drugs in this population and how to address bone fragility in diabetes.

Considering the skeleton a site of diabetic complications, it would benefit from adequate metabolic control. However, this study could not find evidence to support this. In the meta-analysis, there were not enough data to investigate the impact of metabolic control on the risk of fractures. In the clinical study, low bone turnover was also observed in the T1DN- group. In addition, strict metabolic control can increase the risk of hypoglycaemia which increases the risk

of falls and fractures (173, 182). Therefore, adequate metabolic control is advisable and should benefit bone health but the risk of hypoglycaemia should not be neglected(159).

Anti-resorptive drugs are the most common drugs used to treat osteoporosis. They are affordable, widely available and considered safe. Recently concerns were raised about the risk of atypical femur fractures as a rare adverse event in the general population. Diabetes is a risk factor for this adverse event (225). The low bone turnover observed in diabetes associated with the increase in the risk of atypical femur fractures could raise concerns about the use of anti-resorptive drugs in this population. The available information suggests that anti-resorptive drugs are safe in diabetes (186). However, a post hoc analysis from the Freedom trial reported an increase in non-vertebral fractures in the group with diabetes in use of denosumab (226). Although anti-resorptive drugs seem to work in people with diabetes, the population is heterogeneous and we do not know how these drugs affect different subgroups of people with diabetes. It might be possible that people with diabetes could benefit from shorter courses of anti-resorptive drugs. Another possibility is that not all patients with diabetes would respond in the same way and that anti-resorptive drugs would be beneficial for a subgroup of patient with not so low bone turnover, for example. In addition, there is no information about how the concept of drug holiday would apply to this population.

Another suitable option would be to use anabolic drugs. The Dance study is an observational study that assessed real world use of teriparatide (227). It this study the reduction in nonvertebral fracture incidence, increase in BMD (lumbar spine and total hip), and decrease in back pain were similar in T2D and non-diabetic patients in teriparatide treatment. T2D patients had a greater increase in femoral neck BMD. There are no specific data in regards to the use of bisphosphonates after the anabolic treatment in diabetes. There are no specific data if the decrease in the bone turnover observed in diabetes is able to maintain the gain in BMD. Since the mechanisms associated with bone fragility in diabetes are not established any prediction on the behavior of the skeleton in response to drugs is a speculation. More research is needed to investigate the mechanisms associated with bone fragility in diabetes and the efficacy and safety of anti-osteoporotic drugs in this population.

It is likely that non-skeletal features might play an important role in the increased risk of fracture in diabetes. There is data for an increase of falls in diabetes, and there are many features that might contribute to that (93, 150); microvascular complications could impair vision, proprioception and balance increasing the risk of falls (150). In addition, medications associated to hypovolemia, such as SGLT2 could be associated with falls. Furthermore, oral anti-diabetic medications and insulin could be associated with hypoglycemia and also with falls (20).

Limitations

This thesis has limitations. For the systematic reviews, we did not have access to individual data. This limited the analysis of confounders such as age and weight. For the hip and non-vertebral fractures review, we relied on a previous review for the search of the early studies. Considerable amount of data came from registries, with questionable reliability. Some of the studies did not make the distinction between T1D and T2D. There was not enough data to assess the effect of metabolic control, microvascular complications, BMD, falls, hypoglycaemia or the competing risk of death. Non-whites were included, but the majority of the data came from whites. Finally, we found high heterogeneity in most analyses and this should be considered while interpreting the results.

For the clinical study, we were not able to match participants individually. Neuropathy is a broad-spectrum disease and it is possible that participants without neuropathy would have some early stage of the complication. Some participants included had previous history of retinopathy, and therefore, are not free of microvascular disease. This analysis was restricted to DXA and HR-pQCT in a small sample. A broader evaluation of bone health in a greater number of participants would be desirable.

Future directions

We reported the results of bone microarchitecture in T1D but a much broader evaluation would be recommended to try to understand the mechanisms associated with bone fragility in diabetes. This evaluation could include several features such as;

Bone turnover markers could be investigated explore bone turnover in this population. Several other molecules associated with bone health such as vitamin D, PTH could also add useful information.

We speculated that AGEs might have an important role in bone fragility in diabetes, and the assessment of these molecules would be desirable. AGEs can be assessed in blood or urine samples and also in vivo by a device that assess AGE in the skin by measuring skin fluorescence.

We speculated that bone material properties could also be affected. Currently, Osteoprobe can assess bone material properties *in vivo* and could be used to assess bone material properties. There are data in T2D that showed decrease BMSi but there are no data in T1D and no data on the effect of MVD on BMSi.

There are limited data on bone histomorphometry in T1D. A recent study in histomorphometry in T2D that compared participants with and without diabetes and within the diabetic group, participants with poor and good control and also with and without MVD has brought interesting insights (40). However, there is no similar study in T1D.

The low bone turnover in people with diabetes and the increased in the risk of AFF raise concern about the safety of antiresorptive therapies in this population. The analysis of data from reliable databases assessing the incidence of AFF or stress fractures in this population would be desirable.

Finally, there is no specific data on fracture prevention in diabetes. Currently, bone fragility in diabetes is often not detected and when detected is addressed with the same approach used for osteoporosis. However, the pathophysiology of bone fragility in diabetes and osteoporosis seems to be different and this might influence treatment efficacy and safety.

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Appendix

Appendix 1

Literature Searches

Systematic searches of bibliographic databases were conducted to identify reviews and primary studies relating to the risk of bone fractures in patients with diabetes mellitus. Search strategies were developed combining terms for fractures and diabetes mellitus (plus related synonyms) including free-text and thesaurus terms (where available). Search terms were combined using Boolean Operators and database-specific syntax. An initial search was conducted to identify relevant systematic reviews as a source of primary data. Further focused searches were undertaken to identify additional primary studies published since the selected systematic review conducted their searches. Specific details of the individual searches for the reviews and primary studies appear below, including any limits and search filters applies.

1) Searches to identify reviews

Searches to identify systematic reviews relating to the risk of bone fractures in patients with diabetes mellitus were conducted on 7th December 2017 in the following databases:

- Ovid MEDLINE(R) 1946 to November Week 5 2017
- Ovid MEDLINE(R) Epub Ahead of Print December 06, 2017
- Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations December 06, 2017
- Ovid MEDLINE(R) Daily Update December 06, 2017
- Embase via Ovid 1974 to 2017 December 06
- Cochrane Database of Systematic Reviews: Issue 12 of 12, December 2017
- Database of Abstracts of Reviews of Effect via The Cochrane Library: Issue 2 of 4, April 2015
- Health Technology Assessment Database via The Cochrane Library: Issue 4 of 4, October 2016

Searches were limited to humans and English Language where database functionality allowed the application of such limits (Ovid MEDLINE and Embase). The BMJ Best Practice systematic review filter was applied to the MEDLINE and Embase searches[1]. A complete set of search strategies can be found in Appendix 1. The search strategy for reviews was a broad search

including the two conditions from the companion rapid reviews (chronic kidney disease and Parkinson's Disease).

2) Searches to identify primary studies

Searches to identify primary studies relating to the risk of bone fractures in patients with diabetes mellitus were conducted on 28th February 2018 in the following databases:

- Ovid MEDLINE(R) 1946 to February Week 3 2018
- Ovid MEDLINE(R) Epub Ahead of Print February 27, 2018
- Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations February 27, 2018
- Ovid MEDLINE(R) Daily Update February 27, 2018
- Embase via Ovid 1974 to 2018 February 27
- Cochrane Central Register of Controlled Trials: Issue 1 of 12, January 2018

Searches were limited to primary studies added to the databases from June 2006 onwards (where database functionality allowed) based on the search date from the most recent relevant systematic review[2] identified in Search 1 above. The additional limits of humans, English Language, and the exclusion of reviews (identified in Search 1), comments, letters and editorials were applied where possible. In addition to terms relating to bone fractures and diabetes mellitus, terms relating to risk were included in the search to achieve an acceptable balance of precision and recall. A complete set of search strategies can be found in Appendix 2.
The search strategy for primary studies was re-run on Ovid MEDLINE (including Epub Ahead of Print, In-Process & Daily Update) on 29 March 2019. No search limits were applied. To identify studies added to MEDLINE since the original search, the "Create Date" field (.dt) was searched. The search strategy for the update search can be found in Appendix 2.

1.1 Review Search Strategies

MEDLINE

Database: Ovid MEDLINE(R) <1946 to November Week 5 2017>
Search Strategy:
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1 exp Diabetes Mellitus/ or exp Diabetes Mellitus, Type 2/ or exp Diabetes Mellitus, Type 1/ (416094)
2 diabet*.ti,ab. (524556)
3 exp Parkinson Disease/ (64032)
4 parkinson*.ti,ab. (96405)
5 paralysis agitans.mp. (1177)
6 exp Kidney Failure, Chronic/ (94308)
7 (chronic adj2 (kidney* or renal) adj2 (disease* or insufficienc* or failure*)).ti,ab. (63455)
8 (renal adj1 insufficien*).ti,ab. (21779)
9 exp "Chronic Kidney Disease-Mineral and Bone Disorder"/ (3515)
10 "mineral and bone disorder".ti,ab. (529)

11 Renal Osteodystrophy.mp. (2200)
12 (renal adj1 osteodystroph*).ti,ab. (2191)
13 ((end?stage or end stage) adj2 (kidney* or renal*) adj2 (disease* or failure*)).ti,ab. (34642)
14 or/1-13 (819299)
15 exp Fractures, Bone/ (180520)
16 fractur*.ti,ab. (210939)
17 (bone* adj5 (injur* or break* or broken)).ti,ab. (6542)
18 or/15-17 (260692)
19 14 and 18 (5291)
20 limit 19 to (english language and humans) (4246)
21 (review or review,tutorial or review, academic).pt. (2424823)
22 (medline or medlars or embase or pubmed or cochrane).tw,sh. (143959)
23 (scisearch or psychinfo or psycinfo).tw,sh. (16292)
24 (psychlit or psyclit).tw,sh. (935)
25 cinahl.tw,sh. (16843)
26 ((hand adj2 search$) or (manual$ adj2 search$)).tw,sh. (9831)
27 (electronic database$ or bibliographic database$ or computeri?ed database$ or online database$).tw,sh. (23404)
28 (pooling or pooled or mantel haenszel).tw,sh. (79317)
29 (peto or dersimonian or der simonian or fixed effect).tw,sh. (5506)
30 (retraction of publication or retracted publication).pt. (9765)
31 or/22-30 (233649)
32 21 and 31 (128060)
33 meta-analysis.pt. (96815)
34 meta-analysis.sh. (96815)
35 (meta-analys$ or meta analys$ or metaanalys$).tw,sh. (141930)
36 (systematic$ adj5 review$).tw,sh. (108748)
37 (systematic$ adj5 overview$).tw,sh. (1335)
38 (quantitativ$ adj5 review$).tw,sh. (6240)
39 (quantitativ$ adj5 overview$).tw,sh. (244)
40 (quantitativ$ adj5 synthesis$).tw,sh. (1865)
41 (methodologic$ adj5 review$).tw,sh. (4609)
42 (methodologic$ adj5 overview$).tw,sh. (322)
43 (integrative research review$ or research integration).tw. (118)
44 or/33-43 (214243)
45 32 or 44 (264359)
46 20 and 45 (203)

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MEDLINE In-Process, Epub Ahead of Print, Daily Update

Database: Ovid MEDLINE(R) Epub Ahead of Print <December 06, 2017>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations <December 06, 2017>, Ovid MEDLINE(R) Daily Update <December 06, 2017>

Search Strategy:

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1 exp Diabetes Mellitus/ or exp Diabetes Mellitus, Type 2/ or exp Diabetes Mellitus, Type 1/ (1246)
2 diabet*.ti,ab. (63154)
3 exp Parkinson Disease/ (183)
4 parkinson*.ti,ab. (12113)
5 paralysis agitans.mp. (29)
6 exp Kidney Failure, Chronic/ (215)
7 (chronic adj2 (kidney* or renal) adj2 (disease* or insufficienc* or failure*)).ti,ab. (8213)
8 (renal adj1 insufficien*).ti,ab. (1104)
9 exp "Chronic Kidney Disease-Mineral and Bone Disorder"/ (5)
10 "mineral and bone disorder".ti,ab. (130)
11 Renal Osteodystrophy.mp. (146)
12 (renal adj1 osteodystroph*).ti,ab. (126)
13 ((end?stage or end stage) adj2 (kidney* or renal*) adj2 (disease* or failure*)).ti,ab. (4367)
14 or/1-13 (84861)
15 exp Fractures, Bone/ (384)
16 fractur*.ti,ab. (29293)
17 (bone* adj5 (injur* or break* or broken)).ti,ab. (852)
18 or/15-17 (29872)
19 14 and 18 (793)
20 (review or review,tutorial or review, academic).pt. (121998)
21 (medline or medlars or embase or pubmed or cochrane).tw,sh. (31120)
22 (scisearch or psychinfo or psycinfo).tw,sh. (6968)
23 (psychlit or psyclit).tw,sh. (37)
24 cinahl.tw,sh. (3926)
25 ((hand adj2 search$) or (manual$ adj2 search$)).tw,sh. (1762)
26 (electronic database$ or bibliographic database$ or computeri?ed database$ or online database$).tw,sh. (5438)
27 (pooling or pooled or mantel haenszel).tw,sh. (11932)
28 (peto or dersimonian or der simonian or fixed effect).tw,sh. (843)
29 (retraction of publication or retracted publication).pt. (2595)
30 or/21-29 (48293)
31 20 and 30 (12370)
32 meta-analysis.pt. (904)
33 meta-analysis.sh. (904)
34 (meta-analys$ or meta analys$ or metaanalys$).tw,sh. (24212)

35 (systematic$ adj5 review$).tw,sh. (29278)
36 (systematic$ adj5 overview$).tw,sh. (401)
37 (quantitativ$ adj5 review$).tw,sh. (794)
38 (quantitativ$ adj5 overview$).tw,sh. (42)
39 (quantitativ$ adj5 synthesis$).tw,sh. (409)
40 (methodologic$ adj5 review$).tw,sh. (941)
41 (methodologic$ adj5 overview$).tw,sh. (58)
42 (integrative research review$ or research integration).tw. (16)
43 or/32-42 (43000)
44 31 or 43 (47421)
45 19 and 44 (44)

****

EMBASE

Database: Embase <1974 to 2017 December 06>

Search Strategy:

---

1 exp diabetes mellitus/ (819620)

2 exp non insulin dependent diabetes mellitus/ or exp insulin dependent diabetes mellitus/ (284131)

3 diabet*.ti,ab. (787032)

4 exp Parkinson disease/ (130483)

5 parkinson*.ti,ab. (137835)

6 paralysis agitans.mp. (331)

7 exp chronic kidney failure/ (70526)

8 (chronic adj2 (kidney* or renal) adj2 (disease* or insufficienc* or failure*)).ti,ab. (94751)

9 (renal adj1 insufficien*).ti,ab. (28597)

10 exp "chronic kidney disease-mineral and bone disorder"/ (4699)

11 "mineral and bone disorder".ti,ab. (801)

12 exp renal osteodystrophy/ (4564)

13 (renal adj1 osteodystroph*).ti,ab. (2696)

14 ((end?stage or end stage) adj2 (kidney* or renal*) adj2 (disease* or failure*)).ti,ab. (49288)

15 or/1-14 (1266817)

16 exp fracture/ (262538)

17 fractur*.ti,ab. (269255)

18 (bone* adj5 (injur* or break* or broken)).ti,ab. (8897)

19 or/16-18 (350041)

20 15 and 19 (12678)

21 limit 20 to (human and english language) (10608)

22 exp review/ (2380771)

23 (literature adj3 review$).ti,ab. (293127)

24 exp meta analysis/ (138660)

25 exp "Systematic Review"/ (157871)

26 or/22-25 (2615526)

27 (medline or medlars or embase or pubmed or cinahl or amed or psychlit or psyclit or psychinfo or psycinfo or scisearch or cochrane).ti,ab. (201096)

28 RETRACTED ARTICLE/ (8836)

29 27 or 28 (209768)

30 26 and 29 (157826)

31 (systematic$ adj2 (review$ or overview)).ti,ab. (154238)

32 (meta?anal$ or meta anal$ or meta-anal$ or metaanal$ or metanal$).ti,ab. (161788)

33 30 or 31 or 32 (313975)

34 21 and 33 (434)

****

Cochrane Library (CDSR, DARE, HTA)
Last Saved: 07/12/2017 15:09:49.810
ID Search
#1 MeSH descriptor: [Diabetes Mellitus] explode all trees
#2 MeSH descriptor: [Diabetes Mellitus, Type 1] explode all trees
#3 MeSH descriptor: [Diabetes Mellitus, Type 2] explode all trees
#4 diabet*:ti,ab,kw (Word variations have been searched)
#5 MeSH descriptor: [Parkinson Disease] explode all trees
#6 parkinson*:ti,ab,kw
#7 paralysis agitans:ti,ab,kw
#8 MeSH descriptor: [Kidney Failure, Chronic] explode all trees
#9 (chronic near/2 (kidney* or renal) near/2 (disease* or insufficienc* or failure*)):ti,ab,kw
#10 (renal near/1 insufficien*):ti,ab,kw
#11 MeSH descriptor: [Chronic Kidney Disease-Mineral and Bone Disorder] explode all trees
#12 "mineral and bone disorder":ti,ab,kw
#13 Renal Osteodystrophy:ti,ab,kw
#14 (renal near/1 osteodystroph*):ti,ab,kw
#15 ((end?stage or end stage) near/2 (kidney* or renal*) near/2 (disease* or failure*)):ti,ab,kw
#16 {or #1- #15}
#17 MeSH descriptor: [Fractures, Bone] explode all trees
#18 fractur*:ti,ab,kw
#19 (bone* near/5 (injur* or break* or broken)):ti,ab,kw
#20 {or #17-#19}
#21 #16 and #20

Appendix 1.2
Primary Study Search Strategies
MEDLINE
Database: Ovid MEDLINE(R) <1946 to February Week 3 2018>
Search Strategy:
---
1 *Diabetes Mellitus/ or *Diabetes Mellitus, Type 2/ or *Diabetes Mellitus, Type 1/ (210381)
2 diabet*.ti,ab. (472855)
3 *Fractures, Bone/ (44874)
4 fractur*.ti,ab. (193070)
5 (bone* adj5 (injur* or break* or broken)).ti,ab. (6039)
6 or/3-5 (205375)
7 1 or 2 (490336)
8 6 and 7 (2843)
9 limit 8 to (english language and humans) (2280)

10 (200606* or 200607* or 200608* or 200609* or 200610* or 200611* or 200612* or 2007* or 2008* or 2009* or 2010* or 2011* or 2012* or 2013* or 2014* or 2015* or 2016* or 2017* or 2018*).dt. (8295229)
11 9 and 10 (1586)
12 (review or review,tutorial or review, academic).pt. (2215441)
13 (medline or medlars or embase or pubmed or cochrane).tw,sh. (129074)
14 (scisearch or psychinfo or psycinfo).tw,sh. (14868)
15 (psychlit or psyclit).tw,sh. (865)
16 cinahl.tw,sh. (15491)
17 ((hand adj2 search$) or (manual$ adj2 search$)).tw,sh. (8980)
18 (electronic database$ or bibliographic database$ or computeri?ed database$ or online database$).tw,sh. (21132)
19 (pooling or pooled or mantel haenszel).tw,sh. (70838)
20 (peto or dersimonian or der simonian or fixed effect).tw,sh. (4876)
21 (retraction of publication or retracted publication).pt. (8664)
22 or/13-21 (209746)
23 12 and 22 (114932)
24 meta-analysis.pt. (84714)
25 meta-analysis.sh. (84714)
26 (meta-analys$ or meta analys$ or metaanalys$).tw,sh. (124788)
27 (systematic$ adj5 review$).tw,sh. (97811)
28 (systematic$ adj5 overview$).tw,sh. (1228)
29 (quantitativ$ adj5 review$).tw,sh. (5689)
30 (quantitativ$ adj5 overview$).tw,sh. (228)
31 (quantitativ$ adj5 synthesis$).tw,sh. (1715)
32 (methodologic$ adj5 review$).tw,sh. (4175)
33 (methodologic$ adj5 overview$).tw,sh. (292)
34 (integrative research review$ or research integration).tw. (105)
35 or/24-34 (190198)
36 23 or 35 (235348)
37 11 and 36 (109)
38 11 not 37 (1477)
39 COMMENT/ (663372)
40 LETTER/ (925475)
41 EDITORIAL/ (404627)
42 39 or 40 or 41 (1477944)
43 38 not 42 (1445)
44 *RISK/ or *RISK FACTORS/ (4951)
45 *INCIDENCE/ (472)
46 *PREVALENCE/ (720)
47 (risk or inciden* or prevalen* or predict*).ti,ab. (3116451)
48 44 or 45 or 46 or 47 (3118222)
49 43 and 48 (1063)

****

MEDLINE In-Process, Epub Ahead of Print, Daily Update
Database: Ovid MEDLINE(R) Epub Ahead of Print <February 27, 2018>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations <February 27, 2018>, Ovid MEDLINE(R) Daily Update <February 27, 2018>
Search Strategy:
---

1 *Diabetes Mellitus/ or *Diabetes Mellitus, Type 2/ or *Diabetes Mellitus, Type 1/ (668)
2 diabet*.ti,ab. (64842)
3 *Fractures, Bone/ (44)
4 fractur*.ti,ab. (29992)
5 (bone* adj5 (injur* or break* or broken)).ti,ab. (857)
6 or/3-5 (30528)
7 1 or 2 (64869)
8 6 and 7 (614)
9 (200606* or 200607* or 200608* or 200609* or 200610* or 200611* or 200612* or 2007* or 2008* or 2009* or 2010* or 2011* or 2012* or 2013* or 2014* or 2015* or 2016* or 2017* or 2018*).dt. (2851574)
10 (review or review,tutorial or review, academic).pt. (134047)
11 (medline or medlars or embase or pubmed or cochrane).tw,sh. (32543)
12 (scisearch or psychinfo or psycinfo).tw,sh. (6950)
13 (psychlit or psyclit).tw,sh. (43)
14 cinahl.tw,sh. (3982)
15 ((hand adj2 search$) or (manual$ adj2 search$)).tw,sh. (1812)
16 (electronic database$ or bibliographic database$ or computeri?ed database$ or online database$).tw,sh. (5576)
17 (pooling or pooled or mantel haenszel).tw,sh. (12006)
18 (peto or dersimonian or der simonian or fixed effect).tw,sh. (882)
19 (retraction of publication or retracted publication).pt. (2872)
20 or/11-19 (49851)
21 10 and 20 (13179)
22 meta-analysis.pt. (571)
23 meta-analysis.sh. (571)
24 (meta-analys$ or meta analys$ or metaanalys$).tw,sh. (24599)
25 (systematic$ adj5 review$).tw,sh. (30576)
26 (systematic$ adj5 overview$).tw,sh. (391)
27 (quantitativ$ adj5 review$).tw,sh. (800)
28 (quantitativ$ adj5 overview$).tw,sh. (41)
29 (quantitativ$ adj5 synthesis$).tw,sh. (433)
30 (methodologic$ adj5 review$).tw,sh. (966)
31 (methodologic$ adj5 overview$).tw,sh. (52)

32 (integrative research review$ or research integration).tw. (18)
33 or/22-32 (44368)
34 21 or 33 (49160)
35 *RISK/ or *RISK FACTORS/ (6)
36 *INCIDENCE/ (1)
37 *PREVALENCE/ (2)
38 (risk or inciden* or prevalen* or predict*).ti,ab. (515867)
39 35 or 36 or 37 or 38 (515868)
40 8 and 9 (603)
41 40 not 34 (556)
42 41 and 39 (402)

****

EMBASE

Database: Embase <1974 to 2018 February 27>

Search Strategy:

---

1 *diabetes mellitus/ (209761)

2 *non insulin dependent diabetes mellitus/ or *insulin dependent diabetes mellitus/ (171830)

3 diabet*.ti,ab. (787953)

4 or/1-3 (822257)

5 *fracture/ (35807)

6 fractur*.ti,ab. (269828)

7 (bone* adj5 (injur* or break* or broken)).ti,ab. (8909)

8 or/5-7 (281383)

9 4 and 8 (5821)

10 exp review/ (2367906)

11 (literature adj3 review$).ti,ab. (292926)

12 exp meta analysis/ (139554)

13 exp "Systematic Review"/ (159878)

14 or/10-13 (2603229)

15 (medline or medlars or embase or pubmed or cinahl or amed or psychlit or psyclit or psychinfo or psycinfo or scisearch or cochrane).ti,ab. (203676)

16 RETRACTED ARTICLE/ (8904)

17 15 or 16 (212347)

18 14 and 17 (159933)

19 (systematic$ adj2 (review$ or overview)).ti,ab. (154595)

20 (meta?anal$ or meta anal$ or meta-anal$ or metaanal$ or metanal$).ti,ab. (161804)

21 18 or 19 or 20 (315894)

22 9 not 21 (5585)

23 letter/ (954030)

24 editorial/ (569837)

25 23 or 24 (1522748)

26 22 not 25 (5553)

27 *risk factor/ or *risk/ (115621)

28 *incidence/ (7856)

29 *prevalence/ (43211)

30 (risk or inciden* or prevalen* or predict*).ti,ab. (4886202)

31 27 or 28 or 29 or 30 (4894128)

32 26 and 31 (3756)

33 limit 32 to (human and english language) (3046)

34 (200606* or 200607* or 200608* or 200609* or 200610* or 200611* or 200612* or 2007* or 2008* or 2009* or 2010* or 2011* or 2012* or 2013* or 2014* or 2015* or 2016* or 2017* or 2018*).dc. (15219003)

35 33 and 34 (2714)

36 limit 35 to embase (1332)

****

Cochrane Central Register of Controlled Trials
Last Saved: 27/02/2018 11:13:39.099

ID Search
#1 MeSH descriptor: [Diabetes Mellitus] this term only
#2 MeSH descriptor: [Diabetes Mellitus, Type 1] this term only
#3 MeSH descriptor: [Diabetes Mellitus, Type 2] this term only
#4 diabet*:ti,ab
#5 #1 or #2 or #3 or #4
#6 MeSH descriptor: [Fractures, Bone] this term only
#7 fractur*:ti,ab
#8 (bone* near/5 (injur* or break* or broken)):ti,ab
#9 #6 or #7 or #8
#10 #5 and #9 Publication Year from 2006 to 2018

Update Search (conducted 29 March 2019)
Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily 1946 to March 28, 2019
1 *Diabetes Mellitus/ or *Diabetes Mellitus, Type 2/ or *Diabetes Mellitus, Type 1/
2 diabet*.ti,ab.
3 *Fractures, Bone/
4 fractur*.ti,ab.
5 (bone* adj5 (injur* or break* or broken)).ti,ab.
6 or/3-5
7 1 or 2
8 6 and 7
9 (review or review,tutorial or review, academic).pt.
10 (medline or medlars or embase or pubmed or cochrane).tw,sh.
11 (scisearch or psychinfo or psycinfo).tw,sh.
12 (psychlit or psyclit).tw,sh.
13 cinahl.tw,sh.
14 ((hand adj2 search$) or (manual$ adj2 search$)).tw,sh.
15 (electronic database$ or bibliographic database$ or computeri?ed database$ or online database$).tw,sh.
16 (pooling or pooled or mantel haenszel).tw,sh.
17 (peto or dersimonian or der simonian or fixed effect).tw,sh.
18 (retraction of publication or retracted publication).pt.

19 or/10-18
20 9 and 19
21 meta-analysis.pt.
22 meta-analysis.sh.
23 (meta-analys$ or meta analys$ or metaanalys$).tw,sh.
24 (systematic$ adj5 review$).tw,sh.
25 (systematic$ adj5 overview$).tw,sh.
26 (quantitativ$ adj5 review$).tw,sh.
27 (quantitativ$ adj5 overview$).tw,sh.
28 (quantitativ$ adj5 synthesis$).tw,sh.
29 (methodologic$ adj5 review$).tw,sh.
30 (methodologic$ adj5 overview$).tw,sh.
31 (integrative research review$ or research integration).tw.
32 or/21-31
33 20 or 32
34 COMMENT/
35 LETTER/
36 EDITORIAL/
37 34 or 35 or 36
38 *RISK/ or *RISK FACTORS/
39 *INCIDENCE/
40 *PREVALENCE/
41 (risk or inciden* or prevalen* or predict*).ti,ab.
42 38 or 39 or 40 or 41
43 8 and 42
44 33 or 37
45 43 not 44
46 (2018 03* or 2018 04* or 2018 05* or 2018 06* or 2018 07* or 2018 08* or 2018 09* or 2018 10* 2018 11* 2018 12* or 2019*).dt.
47 45 and 46
****

Appendix 2

Data extraction form (Hip and non-vertebral fractures risk in diabetes)

Data extraction field	**Definition**
Study number	Number in spreadsheet, starting with 1
First author (year)	To identify study

Title & biblio	Title and bibliographic information
Study design	Cohort (identifies patients with/without DM, follows forward); Case-control (identifies patients with fractures, looks back); y the definition in the study report; (any doubts, add a comment)
Geographical location	Region, Country
Extracted by	Provide initials
Name of cohort (e.g. MrOS)	This should ideally be a single phrase, not a sentence
Details of database/recruitment process	To include information to allow judgment of representativeness, such as what population the database recruited, coverage of the database for that population, process of contacting and recruiting patients etc
Cases inclusion/exclusion criteria	For case-control studies Cases (people with fractures) For Cohort studies Exposed (people with DM) For case-cohort studies: Cases (people with fractures)
Controls inclusion/exclusion criteria	For case-control studies Controls (people without fractures) For Cohort studies Unexposed (people without DM) For case-cohort studies: Cohort (reference population)
Method of recruitment (e.g. random sample, age matched etc)	Give details for both groups
Method of Diagnosis (definition of DM)	e.g. self reported, medical records, blood tests, registry
Type of diabetes (code)	T1D (type 1 diabetes)

	T2D (type 2 diabetes) All/both Not specified (when the study does not differentiate)
Differential diagnosis T1D vs T2D	(definition of T1D and T2D)
Inclusion criteria relating to Age	e.g. ≥65
Time of Study	Years of recruitment Years of follow-up Case-control studies years of fracture records
Average duration of cohort follow-up	Mean & Range (specify if not full range,e.g. IQR)
Fracture assessment/method of diagnosis	How were fractures ascertained in the study?
Does the study reports number of fractures or number of people with fractures?	Y/N
Has the study excluded people with previous fractures?	Y/N
Sample size (Total N)	This number should be the whole study, not the number in a subgroup.
N patients with DM	
N patients without DM	
N patients with incident fractures	
N patients without fractures	
Ethnicity	If not reported, add notes about probable ethnicity, e.g. if study from Japan, can assume a large proportion are Japanese
Sex at baseline	% Female
Age (years) at enrollment	Mean, error & Range (specify if not full range,e.g. IQR)
Bone mineral density at baseline	mean (error) (range)
Diabetes at baseline	% T1DM %T2DM
Insulin use at baseline	specify categories and provide % If other medication is reported for the DM group, please describe with %
Diabetes duration at baseline	specify categories and provide mean (error) and (range, specify if IQR) or nearest equivalents
Microvascular complications at baseline	specify categories and provide %

BMI at baseline	mean (error) (range)
HbA1c level in diabetics	
Other comments	If need to make any comments
Vertebral fractures reported?	Y/N do not extract the data, just indicate it is available
Subgroups reported?	(Code - one per row) fracture site age range sex diabetes type insulin users diabetes duration microvascular complications BMI
Fracture site subgroup, name fracture site	Name fracture site
Diabetes subgroups	T1D T2D All Other (specify)
Sex subgroups	Male Female All
Age subgroups	Specify, e.g. age range (e.g. 18-40)
Insulin use subgroup	Insulin Non-insulin Both Other (specify)
Diabetes duration subgroup	specify duration or NR
Microvascular complications subgroup	specify type of MV complication
BMI subgroup	specify, e.g. BMI range
N fractures in patients with DM for this subgroup	These numbers relate to the subgroup being reported on this row of the spreadsheet.
Number of people with DM (subgroups)	
N fractures in patients without DM for this subgroup	
Number of patients without DM (subgroups)	
Type of risk estimate reported	HR, OR, IRR etc
Reference group for risk estimate	Which group was the reference group in the multivariable analysis?

Age and sex adjusted risk estimate (95%CI)	If the risk estimates ONLY for age and sex is not available, report the least adjusted (95% CI)
If not only age and sex, report adjustments made	Report which adjustments were made in cases where an age-and-sex-only adjusted analysis was not available
Most fully adjusted risk estimate (95%CI)	
Adjusted variables	List which variables were adjusted for in the analysis
Any additional data adjustments reported	Flagged for information. Where Y, brief details given
Notes	e.g analyses that were excluded from data extraction
Data double checked by second reviewer?	Data checker to add their initials once the data was checked.
Linked studies	Study ID for any studies that may include some or all of the same patients.
Additional comments	Any additional comments that might need to be made.

Appendix 3 Scoring criteria for the Newcastle Ottawa Scales (Case-Control studies and Cohort studies)

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
CASE CONTROL STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?
   a) yes, with independent validation *****
   b) yes, eg record linkage or based on self-reports
   c) no description

2) Representativeness of the cases
   a) consecutive or obviously representative series of cases *****
   b) potential for selection biases or not stated

3) Selection of Controls
   a) community controls *****

b) hospital controls
c) no description

4) Definition of Controls

a) If cases are first occurrence of outcome, then it must explicitly state that controls have no history of this outcome. If cases have new (not necessarily first) occurrence of outcome, then controls with previous occurrences of outcome of interest should not be excluded *

b) No mention of history of outcome

Comparability

1) Comparability of cases and controls on the basis of the design or analysis

a) study controls for age **

b) study controls for gender**

Where an analysis only included one gender, this was considered as an adjustment for gender. Where an analysis reported only a narrow spectrum of age, this was considered as an adjustment for age. Note, all studies in this review will score positively on this item as the selection criteria for the review specified that adjustments for age and sex should have been performed.

Exposure

1) Ascertainment of exposure

a) secure record (eg surgical records) **

b) structured interview where blind to case/control status **

c) interview not blinded to case/control status

d) written self-report or medical record only

e) no description

2) Same method of ascertainment for cases and controls

a) yes **

b) no

3) Non-Response rate

a) same rate for both groups **

b) non-respondents described

c) rate different and no designation

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort
   a) truly representative of the average population with diabetes in the community*****
   b) somewhat representative of the average population with diabetes in the community *****
   c) selected group of users eg nurses, volunteers
   d) no description of the derivation of the cohort

   Note: Downgrade if they excluded people with previous fractures (outcome of interest)

2) Selection of the non-exposed cohort
   a) drawn from the same community as the exposed cohort *****
   b) drawn from a different source
   c) no description of the derivation of the non-exposed cohort

3) Ascertainment of exposure
   a) secure record (eg surgical records) *****
   b) structured interview *****
   c) written self report
   d) no description

4) Demonstration that outcome of interest was not present at start of study
   a) yes *****
   b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis
   a) study controls for age *****
   b) study controls for gender *****

Where an analysis only included one gender, this was considered as an adjustment for gender. Where an analysis reported only a narrow spectrum of age, this was considered as an adjustment for age. Note, all studies in this review will score positively on this item as the selection criteria for the review specified that adjustments for age and sex should have been performed.

Outcome

1) Assessment of outcome
   a) independent blind assessment *****
   b) record linkage *****
   c) self-report
   d) no description

2) Was follow-up long enough for outcomes to occur
   a) yes (1 year or more) *****
   b) no

3) Adequacy of follow up of cohorts
   a) complete follow up - all subjects accounted for *****

b) subjects lost to follow up unlikely to introduce bias - small number lost - > 80 % follow up, or description provided of those lost) *****

c) follow up rate < 80% and no description of those lost

d) no statement

Note: where a study was a comprehensive registry or databases where it could be assumed that emigration was low, a study was scored well for this item.

Appendix 4: Exclusion of full text studies with reasons

Reason for exclusion	**Studies excluded on this basis**
No data on fractures in diabetics	47 (1-47) 1. Agius R, Galea R, Fava S. Bone mineral density and intervertebral disc height in type 2 diabetes. Journal of Diabetes & its Complications. 2016;30(4):644-50. 2. Akeroyd JM, Suarez EA, Bartali B, Chiu GR, Yang M, Schwartz AV, et al. Differences in skeletal and non-skeletal factors in a diverse sample of men with and without type 2 diabetes mellitus. Journal of Diabetes & its Complications. 2014;28(5):679-83. 3. Aleksova J, Wong P, Mulley WR, Choy KW, McLachlan R, Ebeling PR, et al. Serum phosphorus levels and fracture following renal transplantation. Clinical Endocrinology. 2017;87(2):141-8. 4. Armas LA, Akhter MP, Drincic A, Recker RR. Trabecular bone histomorphometry in humans with Type 1 Diabetes Mellitus. Bone. 2012;50(1):91-6. 5. Asokan AG, Jaganathan J, Philip R, Soman RR, Sebastian ST, Pullishery F. Evaluation of bone mineral density among type 2 diabetes mellitus patients in South Karnataka. Journal of Natural Science Biology & Medicine. 2017;8(1):94-8. 6. Barbour KE, Zmuda JM, Boudreau R, Strotmeyer ES, Horwitz MJ, Evans RW, et al. Adipokines and the risk of fracture in older adults. Journal of Bone & Mineral Research. 2011;26(7):1568-76. 7. Bonaccorsi G, Fila E, Messina C, Maietti E, Ulivieri FM, Caudarella R, et al. Comparison of trabecular bone score and hip structural analysis with FRAX in postmenopausal women with type 2 diabetes mellitus. Aging-Clinical & Experimental Research. 2017;29(5):951-7. 8. Carbone LD, Buzkova P, Fink HA, Robbins JA, Bethel M, Isales CM, et al. Association of DPP-4 activity with BMD, body composition, and incident hip fracture: the Cardiovascular Health Study. Osteoporosis International. 2017;28(5):1631-40. 9. Chandran M, Tay D, Tan M, Hao Y, Huang XF, Khoo J, et al. Axial Bmd in Diabetic and Nondiabetic Southeast Asians with Hip Fractures: Do Race and Body Mass Index Matter? Endocrine Practice. 2016;22(6):716-25.

10. Chen W, Simpson JM, March LM, Blyth FM, Bliuc D, Tran T, et al. Co-morbidities only account for a small proportion of excess mortality after fracture: a record linkage study of individual fracture types. Journal of Bone & Mineral Research. 2018;04:04.
11. Chode S, Malmstrom TK, Miller DK, Morley JE. Frailty, Diabetes, and Mortality in Middle-Aged African Americans. Journal of Nutrition, Health & Aging. 2016;20(8):854-9.
12. Colleluori G, Aguirre L, Dorin R, Robbins D, Blevins D, Barnouin Y, et al. Hypogonadal men with type 2 diabetes mellitus have smaller bone size and lower bone turnover. Bone. 2017;99:14-9.
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Appendix 3

Wrist and Ankle Fractures Review Protocol

1. Title of review:
Is the risk of ankle fractures increased in patients with diabetes?

2. Reviewer Contact Details:
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3. Background

Systematic review –Background

Recent studies have suggested that bone could be affected by diabetes. A number of meta-analyses have shown an increased risk of fractures in diabetic populations (Janghorbani et al., 2007, Vestergaard, 2007, Shah et al., 2015). The risk varies depending on the site and type of the disease, the highest reported at the hip for type 1diabetes (DM1) (relative risks –RR- from 3.78 to 6.94) (Shah et al., 2015, Vestergaard, 2007). A number of factors could contribute to this finding: the lack of insulin and its anabolic actions in bone; the frequent early onset of the disease, which could compromise the peak bone mass accrual; the reduced bone mineral density (BMD) observed in DM1 (Pan et al., 2014) and the increased risk of falls related to diabetic complications. However, the role of each of them is still to be defined.
In type 2 diabetes (DM2), the context is different. DM2 patients have increased BMD related mainly to increased body mass index (BMI) (Vestergaard, 2007, Bonds et al., 2006). Weight excess is a risk factor for DM2, and is highly prevalent in this population (2014). In addition, a protective effect against fragility fractures is attributed to weight excess (Johansson et al., 2014). Despite the expected protective effect of weight excess, DM2 patients present an increased risk of fractures (Janghorbani et al., 2007, Vestergaard, 2007). The increase is less than that observed in DM1 (RR 1.38 – 1.7 for hip fracture, for example). However, as DM2 is highly prevalent, it is still remarkable.
The reason for the increased fracture risk observed in DM1 and DM2 is yet to be defined.

Rationale for this review
Ankle fractures are not considered osteoporotic fractures because its occurrence is independent of BMD. High BMI, physical activity and diabetes are risk factors. (Giannini et al., 2013)
On the other hand, distal radius fracture is one of the most common osteoporotic fractures. The incidence is associated with low BMD and increase with age. Although there is a significant immediate morbidity and impact in life quality, there is no increase in mortality.(Porrino et al., 2014)
Although widely used to evaluate fracture risk in the general population, BMD is not effective in detecting the particularities of bone health in diabetic patients, as previously reported. Evaluations of bone microarchitecture by high-resolution peripheral quantitative

computed tomography (HR-pQCT) have shown an increase in cortical porosity in distal radius and tibia, which is more pronounced in the group of diabetic patients with previous fracture (Patsch et al., 2013, Burghardt et al., 2010). In addition, finite element analysis show impairment of stiffness in distal tibia and radius, related to higher cortical porosity (Patsch et al., 2013).
Until now, the increase in cortical porosity is the main structural abnormality observed in bone in diabetic patients. As the distal region of long bones such as the radius and tibia are rich in cortical bone, an increase in fracture rates would be expected at these sites in the diabetic population as a . clinical effect of this structural abnormality.
The aim of this review is to identify if there is an increase in the risk of radius and tibia fractures in patients with diabetes

4. Focused review question

Question:
Is the risk of ankle and radius fractures increased in patients with diabetes?

Population: Adults
Intervention: -> epidemiological study -> exposure: diabetes (type 1 and type 2)
Comparators: non diabetic patients
Outcomes: ankle fractures

5. Search Strategy
Search terms

Terms	**Thesaurus**	**Free-text**
Population	Adults	
Intervention (s) Exposure	Diabetes mellitus	Type 1 diabetes Type 2 diabetes
Outcomes	Ankle fractures	Ankle fractures
Comparators	NA	NA

Search Limits

Study designs	Cohort studies Case control studies Registry studies Epidemiological studies
Publication types	Primary studies
Date of publication	No limits
Language	No limits
Other limits	

Sources to be searched

Databases	Medline (via Ovid) LILACS The Cochrane Library EMBASE
Grey literature	Abstracts from related meetings ASBMR 2015
Other sources	References list from relevant related papers

6. Study Selection
Inclusion and exclusion criteria

Selection Criteria	**Inclusion**	**Exclusion**

Population	Adults: -Male and female>18 years old;	Studies that include data of children fracture; Studies that do not specify the age group;
Intervention	Patients with diabetes: -Type 1 or type 2; -Diagnosis stablished by self report, medical records, or exams results according to WHO or ADA diabetes diagnostic criteria	Studies that uses different diagnosis criteria for diabetes; Studies lacking a clear condition of the medical condition of interest;
Comparators	Male and female adult patients without diabetes	Studies without a comparison non diabetic group;
Outcomes	Occurrence of ankle fracture Diagnosis criteria: -Self report; medical records (report or radiology exams)	Studies reporting predicted fracture risk based on an algorithm or risk tool; Studies that report mainly traumatic or high energy fracture.
Language	English, French, Portuguese, Spanish	Other languages
Other		Studies lacking data or mean to calculate OR/RR and CI

7. Quality assessment strategy

There is no standard international tool to evaluate quality control in observational studies. Review papers suggest to avoid the use of scales, and to prefer the use of checklists with comments.

Features to be addressed:

DM diagnosis

Fracture diagnosis

Type of study

Patients selection

Control of confounders

Statistical analysis

In order to try to evaluate most of these aspects, the Newcastle-Ottawa quality assessment tool will be used.

8. Data Extraction

Data will be extracted by one reviewer (with no blinding to authors or journal) using a data extraction form (google forms). Any doubts will be discussed with a supervisor.

Should multiple publications of the same study be identified, data will be extracted and reported from a single one.

Data items to be extracted:

- Author, year;
- Study design;
- Population size and characteristics;
- Relevant clinical features;
- Additional factors that may affect the risk of fractures or falls;
- Mode by which the fracture occurred;
- Fracture site;
- Fracture diagnostic methods;
- Risk of fracture reported as relative risk (RR) or odds ratio (OR);

9. Proposed Data synthesis

A narrative review will be performed. If the data collected is sufficient, a meta-analysis will be performed.

10. Review Timetable

Task	**Completion date**
Focus question	29/02/16
Draft protocol	15/03/16

Scoping search	16/03/16
Final protocol	30/03/16
Full searches	30/03/16
Order papers	30/03/16
Study selection	13/04/16
Quality assessment	27/04/16
Data extraction	27/04/16
Data synthesis	25/04/16
Draft review submission	01/06/16
Final review submission	08/06/16

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