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Full Length Article

The association between insulin use and volumetric bone mineral density, bone micro-architecture and bone strength of the distal radius in patients with type 2 diabetes – The Maastricht study



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ABSTRACT

Type 2 diabetes mellitus (T2DM) has been associated with an increased risk of fractures, despite normal to increased bone mineral density (BMD). Insulin use is one of the factors linked to this increased fracture risk. However, direct negative effects of insulin on bone quality are not expected since insulin is thought to be anabolic to bone. In this cross-sectional study the association between insulin use and volumetric BMD (vBMD), bone micro-architecture and bone strength of the distal radius, as measured with HR-pQCT, was examined. Data from 50 participants with T2DM of The Maastricht Study (mean age 62 ± 7.5 years, 44% women) was used. Participants were classified as insulin user ($n = 13$) or non-insulin user ($n = 37$) based on prescription data. Linear regression analysis was used to estimate the association between current insulin use and HR-pQCT derived parameters. After adjustment for age, sex, body mass index, glycated hemoglobin A1c and T2DM duration, insulin use was associated with lower total vBMD (standardized beta (β): -0.56 (95% CI: -0.89 to -0.24)), trabecular vBMD (β : -0.58 (95% CI: -0.87 to -0.30)), trabecular thickness (β : -0.55 (95% CI: -0.87 to -0.23)), cortical thickness (β : -0.41 (95% CI: -0.74 to -0.08)), log cortical pore volume (β : -0.43 (95% CI: -0.73 to -0.13)), bone stiffness (β : -0.39 (95% CI: -0.62 to -0.17)) and failure load (β : -0.39 (95% CI: -0.60 to -0.17)) when compared to the non-insulin users. Insulin use was not associated with cortical vBMD, trabecular number, trabecular separation, cortical porosity and cortical pore diameter. This study indicates that insulin use is negatively associated

Abbreviations: aBMD, areal bone mineral density; AGE, advanced glycation end product; ATC, anatomical therapeutical chemical; β , standardized beta; BMD, bone mineral density; CI, confidence interval; HbA1c, glycated hemoglobin A1c; HR-pQCT, high resolution peripheral quantitative tomography; OGTT, oral glucose tolerance test; T2DM, type 2 diabetes mellitus; vBMD, volumetric bone mineral density.

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with bone density, bone micro-architectural and bone strength parameters. These findings may partly explain the previously observed increased fracture risk in insulin users, although there may be residual confounding by other factors related to disease severity in insulin users.

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1. Introduction

Type 2 diabetes mellitus (T2DM) is a highly prevalent chronic disease leading to complications such as neuropathy, retinopathy and nephropathy [1]. More recently, T2DM has been associated with an increased risk of fractures, despite a normal to increased bone mineral density (BMD) [2,3]. The mechanisms leading to this increased fracture risk are not completely elucidated, but both an increased falling frequency and bone fragility are thought to contribute to the increased fracture risk [4,5]. Bone fragility can be the result of various factors, and in patients with T2DM, among others, unfavorable changes in micro- and macro-architecture of the bone, accumulation of advanced glycation end products (AGEs) in bone collagen and a low bone turnover have been reported [4,5].

The use of antihyperglycemic drugs may also contribute to the increased fracture risk. Except for thiazolidinediones, oral antihyperglycemic drugs are not associated with an increased fracture risk [6,7]. However, insulin has been associated with an increased fracture risk [7]. Since previous studies showed that insulin may be anabolic to bone [8] it has been hypothesized that the increased fracture risk in insulin users is not caused by the drug itself. It is rather due to an increased falling frequency and to the long-term negative effects of hyperglycemia on bone quality, as insulin is most often used in patients with long disease duration.

The association between hyperinsulinemia and areal BMD (aBMD) in nondiabetic participants, as measured with DXA, has been examined in several studies that demonstrated a positive association between hyperinsulinemia and aBMD [9–11]. However, only two small studies have examined the association between insulin therapy and aBMD in patients with T2DM. Both showed a positive correlation between insulin dose and aBMD [12,13].

High resolution peripheral quantitative computed tomography (HR-pQCT) is a relatively new technique which can be used to measure volumetric BMD (vBMD), micro-architecture and bone strength [14,15]. The association between insulin use and bone parameters measured by HR-pQCT has not been studied before [7]. Therefore, the aim of this study was to examine the association between insulin use and vBMD, bone micro-architecture and bone strength in participants with T2DM. It is hypothesized that insulin use will be positively associated with HR-pQCT derived parameters when compared to non-insulin use.

2. Materials and methods

2.1. Source population

Data from The Maastricht Study, an ongoing observational prospective population-based cohort study, was used in the present study. The rationale and methodology have been described previously [16]. In brief, the study focuses on the etiology, pathophysiology, complications and comorbidities of T2DM and is characterized by an extensive phenotyping approach. Eligible participants were all individuals aged between 40 and 75 years and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns as well as from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known T2DM status, with an oversampling of individuals with T2DM, for reasons of efficiency.

To determine glucose metabolism status, all participants, except those who used insulin, underwent a standardized 2-h 75 g oral glucose

tolerance test (OGTT) after an overnight fast. For safety reasons, participants with a fasting glucose level > 11.0 mmol/l (> 200.0 mg/dl), as determined by a capillary blood glucose measurement, did not undergo the OGTT. Fasting glucose level, 2-h plasma glucose level and information about diabetes medication were used to determine glucose metabolism status. Participants were classified as having T2DM when they had a fasting plasma glucose level ≥ 7.0 mmol/l (≥ 126 mg/dl) or a two hour plasma glucose level ≥ 11.1 mmol/l (≥ 200 mg/dl) as specified by the World Health Organization guidelines [17] or if they used antihyperglycemic drugs at baseline. Individuals without type 1 diabetes who used antihyperglycemic drugs were classified as having T2DM. Participants who were not classified as T2DM, but did use an antihyperglycemic drug in the six months prior to the date of the HR-pQCT scan (based on their pharmacy data) were also included.

The present study includes cross-sectional data from participants with T2DM who completed the baseline survey between November 2010 and September 2013 and returned to the research center between March 2015 and February 2016 for the HR-pQCT scan of the distal radius. Dispensing records were collected at the pharmacy for all participants who gave written informed consent for the collection of their drug dispensing history. Dispensing data was available from January 1st 1991 through the date of the HR-pQCT scan and contained the product name, the anatomical therapeutic chemical (ATC) code [18], the dispensed quantity, the dispensing date and the prescribed daily dose [19]. When a participant had a prescription for insulin (ATC code A10A) in the six months before the date of the HR-pQCT scan, the participant was classified as current insulin user. All other T2DM participants were classified as non-insulin users. The mean time since first prescription of insulin was calculated from the prescription data.

The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of the Netherlands (Permit 131,088-105,234-PG). All participants gave written informed consent.

2.2. HR-pQCT imaging

The non-dominant radius was scanned on an HR-pQCT scanner (Xtreme-CT; Scanco Medical AG, Brüttisellen, Switzerland) using the standard in vivo protocol as described in literature [20,21]. If the patient has previously sustained a distal radius fracture at the non-dominant site, the dominant site was scanned. The forearm was placed into a carbon fiber cast. An anteroposterior scout projection of the scan site was acquired for positioning of the tomographic acquisition. A reference line was placed on the radial joint surface. The scan volume spanned 9.02 mm in length and started 9.5 mm from the reference line in the proximal direction. Images were reconstructed using an isotropic voxel size of 82 μm , resulting in 110 consecutive slices. Total scan time was 2.8 min, with each acquisition resulting in an effective dose of approximately 3 μSv . All scans were graded with regard to motion, and scans with quality 4 or 5 were repeated once [22]. Only scans with quality 1 to 3 were used for subsequent image analysis [23].

2.3. Image analysis of HR-pQCT scans

All scans were evaluated using the standard patient evaluation protocol that was provided by the manufacturer and that has been described previously in detail [24–26]. First, the periosteal contour was automatically derived and manually modified when contours visually deviated from the periosteal boundary [27]. The images were

automatically segmented and the following bone density parameters were calculated from the images: total vBMD, trabecular vBMD and cortical vBMD. In the trabecular region, the micro-architectural parameters trabecular number, trabecular thickness and trabecular separation were calculated. For the cortical region, cortical thickness was calculated. In addition, extended analysis of the cortical compartment was performed to obtain cortical pore volume, cortical porosity and mean cortical pore diameter [28]. Cortical pore volume was calculated as the volume of all voxels identified as intracortical pore space. Cortical porosity was calculated as the ratio of the cortical pore volume to the total volume of the cortical compartment.

Micro-finite element analysis was performed by creating micro-finite element models directly from the segmented HR-pQCT images as described previously [29,30]. In short, all voxels representing bone tissue were converted into brick elements of the same size. A Young modulus of 10 GPa and a Poisson ratio of 0.3 were assigned to every element. Compression stiffness and estimated failure load were determined by applying a virtual “high-friction” compression test in the axial direction [29].

2.4. Covariates

All covariates were determined at the baseline visit between November 2010 and September 2013. Weight and height were measured without shoes and wearing light clothing using a scale and stadiometer to the nearest 0.5 kg or 0.1 cm (Seca, Hamburg, Germany). BMI was calculated by dividing weight in kilogram by height in meters squared. HbA1c level and creatinine level were determined as described elsewhere [16]. Alcohol use, smoking status (never, former or current), a history of a fracture at or above the age of 50 and T2DM duration were recorded at the baseline visit [18]. Alcohol consumption was classified into three categories: non-consumers, low consumers (≤ 7 glasses per week for women and ≤ 14 glasses per week for men), and high consumers (> 7 glasses per week for women and > 14 glasses per week for men).

For the current study, the time between the baseline visit and the date of the HR-pQCT scan was added to the T2DM duration at baseline. If T2DM duration was not available, it was estimated by the time between the first antihyperglycemic prescription and the date of the HR-pQCT scan. Use of other antihyperglycemic drugs in the six months before the date of the HR-pQCT scan was determined by the prescription data using ATC codes: metformin (ATC code A10BA02, A10BD02,03,05,07,08,10,11,13–18), sulfonylurea derivative (ATC code A10BB, A10BD01, A10BD02), dipeptidyl peptidase 4 inhibitor (ATC code A10BH), thiazolidinedione (A10BG) or other blood glucose lowering drugs (A10BX). Use of anti-osteoporotic drugs and use of systemic corticosteroids in the six months before the date of the HR-pQCT scan was determined by the prescription data using ATC codes: drugs affecting bone structure and mineralization (M05B) and corticosteroids for systemic use (H02).

2.5. Statistical analysis

General characteristics were compared between the insulin and non-insulin users. The independent student's *t*-test or the Mann-Whitney *U* test was used to test for significant differences of continuous normal or not-normal distributed variables and a chi-square test for differences in categorical variables. Log-transformation was performed if variables showed a skewed distribution. Multiple linear regression analysis was used to estimate the association between current use of insulin and HR-pQCT derived parameters as compared to the non-insulin users, yielding standardized beta's (β) and 95% confidence intervals (CIs). The same models were used for all regression analyses: model 1 was adjusted for age and sex, model 2 was additionally adjusted for BMI, HbA1c and duration of diabetes. A *p*-value < 0.05 was considered

statistically significant and analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

In total, data from 59 T2DM participants who had a HR-pQCT scan and pharmacy dispensing data available was retrieved. Data of eight participants were excluded due to extreme motion artifacts of the HR-pQCT scan (grade 5 $n = 2$, grade 4 $n = 6$) and data from one participant was excluded because of extreme outliers. From the 50 included participants, 13 were classified as insulin user and 37 were classified as non-insulin user.

The general characteristics and the mean HR-pQCT derived bone parameters of the non-insulin and insulin users are shown in Table 1. The non-insulin users were slightly older, had a shorter time since first hyperglycemic prescription, a shorter duration of diabetes and lower mean HbA1c levels. There was no significant difference in the number of fractures at or above the age of 50 and the use of anti-osteoporotic drugs between the groups. Volumetric bone mineral density was lower in the insulin users when compared to the non-insulin users (total vBMD -67.6 mgHA/cm³, $p < 0.01$; trabecular vBMD -30.3 mgHA/cm³, $p = 0.03$; cortical vBMD -54.16 mgHA/cm³, $p = 0.06$). Except for trabecular thickness being significantly lower in the insulin users (-0.01 mm, $p < 0.01$), there were no differences in the trabecular micro-architecture between the groups. The cortical micro-architecture of the insulin users was unfavorable compared to the non-insulin users (cortical thickness -0.20 mm, $p = 0.02$; cortical pore volume -5.80 mm³, $p = 0.03$; cortical porosity -2.38% , $p = 0.38$; cortical pore diameter -0.01 mm, $p = 0.08$). Bone strength was also lower in the insulin users (bone stiffness -18.39 kN/mm, $p = 0.04$; failure load -815.41 N, $p = 0.05$) when compared to the non-insulin users.

The association between insulin use and HR-pQCT derived bone parameters is shown in Table 2. After adjustment for age, sex, BMI, HbA1c and diabetes duration (model 2), current insulin use was associated with parameters of volumetric bone mineral density, bone micro-architecture and bone strength (volumetric bone mineral density: lower total vBMD ($\beta -0.56$ (95% CI -0.89 – -0.24)), trabecular vBMD ($\beta -0.58$ (95% CI -0.87 – -0.30)); micro-architecture: trabecular thickness ($\beta -0.55$ (95% CI -0.87 – -0.23)), cortical thickness ($\beta -0.41$ (95% CI -0.74 – -0.08)), cortical pore volume ($\beta -0.43$ (95% CI -0.73 – -0.13)); strength: bone stiffness ($\beta -0.39$ (95% CI -0.62 – -0.17)), failure load ($\beta -0.39$ (95% CI -0.60 – -0.17))) as compared to the non-insulin users. Current insulin use was not associated with cortical vBMD, trabecular number, trabecular separation, cortical porosity and cortical pore diameter.

4. Discussion

The present study examined the association between current insulin use and HR-pQCT derived bone parameters in patients with T2DM. Literature on the association between insulin therapy and BMD in T2DM patients is scarce and there are no studies available that examined the effects of insulin therapy on bone micro-architecture or bone strength. Only two small studies have examined the association between insulin dose and BMD, and both found a positive association [12,13]. In contrast to these studies as well as our own hypothesis, the present study shows that current insulin use was associated with lower total vBMD, trabecular vBMD, trabecular thickness, cortical thickness, cortical pore volume, bone stiffness and failure load as compared to non-insulin users after adjustment for potential confounders.

In previous studies it was reported that insulin use is associated with an increased fracture risk in T2DM patients [7]. The mechanisms leading to this increased fracture risk are not completely understood, but a direct negative effect of insulin use of bone was thought to be unlikely because in-vitro studies showed that insulin is an anabolic bone agent [8].

Table 1
General characteristics of the study population.

	Non-insulin users (n = 37)	Insulin users (n = 13)	p-value
Age, years	62.9 (7.6)	60.4 (5.9)	0.50
Female	15 (40.5)	6 (46.2)	0.72
BMI, kg/m ²	30.3 (5.0)	30.9 (5.4)	0.71
Smoking status			
Current	6 (16.2)	0 (0)	
Former	17 (45.9)	10 (76.9)	0.08
Never	13 (35.1)	2 (15.4)	
Missing	1 (2.7)	1 (7.7)	
Alcohol use			
None	6 (16.2)	5 (38.4)	
Low	20 (54.1)	4 (30.8)	0.19
High	10 (27.0)	3 (23.1)	
Missing	1 (2.7)	1 (7.7)	
Time since first antihyperglycemic prescription, years	4.1 [5.0]	8.5 [8.3]	<0.01
Time since first insulin prescription, years	n/a	7.0 [4.7]	n/a
Use of drugs six months prior to the scan			
Metformin	26 (70.3)	8 (61.5)	0.56
Sulfonylurea derivatives	6 (16.2)	0 (0)	0.12
Dipeptidyl peptidase 4 inhibitors	0 (0)	2 (15.4)	<0.01
Thiazolidinediones	0 (0)	0 (0)	n/a
Other antihyperglycemic drugs	0 (0)	0 (0)	n/a
Systemic corticosteroids	2 (5.4)	1 (7.7)	0.77
Anti-osteoporotic drugs	1 (2.7)	1 (7.7)	0.43
History of a fracture ≥ 50 years of age	5 (13.5)	0 (0)	0.16
Duration of diabetes, years	4.1 [3.2]	14.7 [6.0]	<0.01
HbA1c, %	6.6 (0.7)	7.7 (0.8)	<0.01
Creatinine, $\mu\text{mol/L}$	77.5 (19.6)	79.9 (17.4)	0.70
Quality grade of HR-pQCT scan			
1	8 (21.1)	3 (23.1)	
2	21 (55.3)	6 (46.2)	0.87
3	9 (23.7)	4 (30.8)	
HR-pQCT derived bone parameters			
Bone mineral density			
Total vBMD, mgHA/cm ³	326.2 (75.6)	258.6 (61.0)	<0.01
Trabecular vBMD, mgHA/cm ³	173.1 (44.2)	142.9 (32.8)	0.03
Cortical vBMD, mgHA/cm ³	854.0 (82.9)	799.9 (95.5)	0.06
Bone micro-architecture			
Trabecular number, mm ⁻¹	1.95 (0.37)	1.95 (0.35)	0.98
Trabecular thickness, mm	0.07 (0.01)	0.06 (0.01)	<0.01
Trabecular separation, mm	0.47 (0.17)	0.47 (0.10)	0.99
Cortical thickness, mm	0.84 (0.26)	0.64 (0.24)	0.02
Cortical pore volume, mm ³	19.10 (8.76)	13.30 (5.79)	0.03
Cortical porosity, %	3.36 (1.21)	2.98 (1.64)	0.38
Cortical pore diameter, mm	0.18 (0.02)	0.17 (0.02)	0.08
Bone strength			
Bone stiffness, kN/mm	100.5 (27.7)	82.1 (23.3)	0.04
Failure load, N	4796.5 (1298.2)	3981.1 (1101.5)	0.05

Continuous variables are presented as mean (SD) or median [IQR], categorical variables as number of participants (%). P-values in bold are statistically significant. Abbreviations: HbA1c, glycated hemoglobin A1c; HR-pQCT, high resolution peripheral quantitative computed tomography; n/a, not applicable; vBMD, volumetric bone mineral density.

It was proposed that insulin use may be a surrogate for disease severity and disease duration as insulin is most often used by T2DM patients with long disease duration. The observed increased fracture risk in insulin users may therefore be due to factors associated with the disease itself. For example, complications of T2DM and risk factors for falling such as diabetic neuropathy and retinopathy are more common in patients with long disease duration, and one of the proposed mechanisms of the increased fracture risk in insulin users was therefore an increased falling frequency. Additionally, it may be hypothesized that insulin users are generally more insulin resistant than non-insulin users. Insulin resistance may result in impaired insulin signaling in osteoblasts, which leads to impaired bone micro-architecture due to deteriorated osteoblast proliferation, differentiation and survival [31]. Insulin resistance may therefore be another mechanism leading to the observed increased

Table 2
The association between insulin use and HR-pQCT derived bone parameters.

	Model 1 (β (95% CI))	Model 2 (β (95% CI))
Bone mineral density		
Total vBMD, mgHA/cm ³	-0.39 (-0.65--0.13)*	-0.56 (-0.89--0.24)*
Trabecular vBMD, mgHA/cm ³	-0.29 (-0.55--0.04)*	-0.58 (-0.87--0.30)*
Cortical vBMD, mgHA/cm ³	-0.30 (-0.56--0.05)*	-0.28 (-0.61--0.05)
Bone micro-architecture		
Trabecular number, mm ⁻¹	0.00 (-0.27--0.27)	-0.28 (-0.59--0.02)
Trabecular thickness, mm	-0.41 (-0.66--0.16)*	-0.55 (-0.87--0.23)*
Trabecular separation, mm	0.00 (-0.26--0.27)	0.23 (-0.09--0.55)
Cortical thickness, mm	-0.34 (-0.60--0.08)*	-0.41 (-0.74--0.08)*
Cortical pore volume, mm ³	-0.29 (-0.52--0.05)*	-0.43 (-0.73--0.13)*
Cortical porosity, %	-0.08 (-0.32--0.17)	-0.20 (-0.51--0.12)
Cortical pore diameter, mm	-0.24 (-0.50--0.03)	-0.26 (-0.61--0.08)
Bone strength		
Bone stiffness, kN/mm	-0.27 (-0.45--0.08)*	-0.39 (-0.62--0.17)*
Failure load, N	-0.25 (-0.43--0.07)*	-0.39 (-0.60--0.17)*

The analysis included 50 participants; 37 non-insulin users (reference group), and 13 insulin users. Model 1: adjusted for age and sex. Model 2: model 1 + BMI, glycated hemoglobin A1c and duration of diabetes. Cortical pore volume is log transformed.

* Statistically significant, $p < 0.05$. Abbreviations: vBMD, volumetric bone mineral density.

fracture risk in insulin users. Alternatively, a study in patients with T2DM showed beta-cell decay over time [32], which leads to a decrease in the endogenous insulin production and contributes negatively to bone quality. In our study, the diabetes duration of the insulin users was longer than the non-insulin users (14.7 vs 4.1 year, respectively), and hypoinsulinemia may thus be more prominently present in the insulin users, while substitution with exogenous insulin may not completely mimic the effects of endogenous insulin. Furthermore, the cumulative effect of chronic hyperglycemia will be larger in insulin users than in non-insulin users. Long-term hyperglycemia may have several negative effects on bone architecture, such as an increased formation and accumulation of AGE's, which result in impaired bone collagen quality and thereby decreased bone strength [33]. Elevated blood glucose levels also lead to hypercalcuria [34], which may influence bone mineralization. Finally, insulin users may be sarcopenic more often than non-insulin users [35]. As muscle strength influences bone strength, this may be another mechanism resulting in an increased fracture risk.

Although the results in this study are adjusted for HbA1c and disease duration, which were used as proxies for effect of the disease itself, there may still be residual confounding by other factors related to disease severity. Further information regarding disease severity such as the presence of micro- and macrovascular complications will be very useful to take into account, but for this study we did not have this information. Additionally, we had no HR-pQCT data of participants with an impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). Inclusion of a group with IFG or IGT could help to test some of the above mentioned hypotheses about the mechanism whereby insulin use is negatively associated with HR-pQCT derived bone parameters.

Previous studies showed that T2DM is a low bone turnover condition, as both markers of bone formation and bone resorption are lower in patients with T2DM than in healthy subjects [36,37]. It may be hypothesized that changes in bone micro-architecture may be mediated by changes in bone turnover markers, i.e. unfavorable vBMD, bone micro-architecture and bone strength such as observed in our study are accompanied by or preceded by an increase in bone resorption markers while the bone formation markers are stable or decreased. Although no literature is available on the effect of exogenous insulin substitution on bone turnover markers, a study by Basu et al. found no alterations in bone turnover markers during physiological changes in insulin levels [38]. Additionally, higher levels of serum insulin were associated with increased levels of the bone turnover markers [39], decreased levels of bone resorption markers, and increased vBMD of the subtrocantalic

femur on low-resolution CT [40]. Unfortunately, no data on bone turnover markers was available in our study either. Future studies examining the association between insulin use and HR-pQCT derived bone parameters should also measure bone turnover markers to verify this hypothesis. Furthermore, DXA scans of the participants were not available. Due to the small number of participants we were not able to adjust for potential confounders such as use of glucocorticoids, a history of a fracture at or above the age of 50, a parental history of hip fracture, vascular complications of diabetes and menopausal status. Within the non-insulin user group there were some users of glucocorticoids, and use of them has been associated with increased fracture risk [42] and this might have confounded the investigated association. Finally, we used an automatic instead of a semi-automatic algorithm to calculate cortical bone parameters. It has been argued that on top of a trabecular and cortical region, a transitional zone (the region between the trabecular and cortical region) should be identified [43,44]. In this manuscript, the endocortical contour was not manually adjusted to prevent intra-operator variability, and this may have resulted in over- or underestimation of the cortical bone parameters.

This study has some limitations. First, this study has a cross-sectional design and because of this design we can only speculate about the mechanisms whereby insulin use is associated with impaired bone quality. Second, because special clearance for radiological examinations within The Maastricht Study by the Dutch Ministry of Health was required, HR-pQCT measurements started later during the course of the study. Therefore, HR-pQCT data were available for a relatively small number of participants resulting in reduced power of the study which may have led to over- or underestimation of the associations [41]. The present results should therefore be interpreted with caution. HR-pQCT scans of the distal tibia were only recently allowed within the Maastricht Study and therefore these scans were not available in our study population. Since the distal tibia is a weight bearing bone, in contrast to the distal radius, the association between insulin use and HR-pQCT derived bone parameters at the distal tibia may be different and is subject for future analyses. Additionally, no information on vitamin D and parathyroid hormone levels were available for the study population and therefore patients with secondary osteoporosis may have been included.

In conclusion, in this cross-sectional study we found that insulin use was negatively associated with bone mineral density, bone micro-architectural and bone strength parameters at the distal radius measured with HR-pQCT in T2DM patients. Therefore, the previously observed increased fracture risk in insulin users might be partly due to bone fragility, although there may be residual confounding by other factors related to disease severity in insulin users. Replication of these findings in larger, preferably longitudinal studies is needed.

Authors' roles

Study design: EdW, JD, FdV and JvdB. Data collection: EdW, JD, RH, MS, PD, CvdK, SS, CS, NS, AK. Data analysis: EdW and JD. Data interpretation: EdW, AD, JdJ, FdV, JvdB. Drafting manuscript: EdW, AD, FdV, JvdB. Revising manuscript content: All authors. Approving final version of manuscript: All authors. EdW and AD take responsibility for the integrity of the data analysis.

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Declaration of interest

Conflicts of interest

None.

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References

- [1] G. Danaei, M.M. Finucane, Y. Lu, G.M. Singh, M.J. Cowan, C.J. Paciorek, et al., National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants, *Lancet* 378 (9785) (2011) 31–40.
- [2] A.V. Schwartz, D.E. Sellmeyer, K.E. Ensrud, J.A. Cauley, H.K. Tabor, P.J. Schreiner, S.A. Jamal, D.M. Black, Cummings SR; study of osteoporotic features research group. Older women with diabetes have an increased risk of fracture: a prospective study, *J. Clin. Endocrinol. Metab.* 86 (1) (2001) 32–38.
- [3] P. Vestergaard, Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis, *Osteoporos. Int.* 18 (4) (2007) 427–444.
- [4] E.A. de Waard, T.A. van Geel, H.H. Savelberg, A. Koster, P.P. Geusens, J.P. van den Bergh, Increased fracture risk in patients with type 2 diabetes mellitus: an overview of the underlying mechanisms and the usefulness of imaging modalities and fracture risk assessment tools, *Maturitas* 79 (3) (2014) 265–274.
- [5] N. Napoli, M. Chandran, D.D. Pierroz, B. Abrahamsen, A.V. Schwartz, S.L. Ferrari, Mechanisms of diabetes mellitus-induced bone fragility, *Nat. Rev. Endocrinol.* 13 (4) (2017) 208–219.
- [6] A. Palermo, L. D'Onofrio, R. Eastell, A.V. Schwartz, P. Pozzilli, N. Napoli, Oral anti-diabetic drugs and fracture risk, cut to the bone: safe or dangerous? A narrative review, *Osteoporos. Int.* 26 (8) (2015) 2073–2089.
- [7] M.P. Gilbert, E. Pratley, The impact of diabetes and diabetes medications on bone health, *Endocr. Rev.* 36 (2) (2015) 194–213.
- [8] K.M. Thrallkill, C.K. Lumpkin Jr., R.C. Bunn, S.F. Kemp, J.L. Fowlkes, Is insulin an anabolic agent in bone? Dissecting the diabetic bone for clues, *Am. J. Physiol. Endocrinol. Metab.* 289 (5) (2005) E735–E745.
- [9] R.P. Stolk, P.L. van Daele, H.A. Pols, H. Burger, A. Hofman, J.C. Birkenhäger, S.W. Lamberts, D.E. Grobbee, Hyperinsulinemia and bone mineral density in an elderly population: the Rotterdam Study, *Bone* 18 (6) (1996) 545–549.
- [10] E. Barret-Conner, D. Kritiz-Silverstein, Does hyperinsulinemia preserve bone? *Diabetes Care* 19 (1996) 1388–1392.
- [11] I.R. Reid, M.C. Evans, G.J.S. Cooper, R.W. Ames, J. Stapleton, Circulating insulin levels are related to bone density in postmenopausal women, *Am. J. Physiol. Endocrinol. Metab.* 265 (1993) E655–E659.
- [12] Y. Fukunaga, J. Minamikawa, D. Inoue, H. Koshiyama, Does insulin use increase bone mineral density in patients with non-insulin-dependent diabetes mellitus? *Arch. Intern. Med.* 157 (1997) 2668–2669.
- [13] R.S. Weinstock, R.S. Goland, E. Shane, T.L. Clemens, R. Lindsay, J.P. Bilezikian, Bone mineral density in women with type II diabetes mellitus, *J. Bone Miner. Res.* 4 (1989) 97–101.
- [14] P. Geusens, R. Chapurlat, G. Schett, A. Ghasem-Zadeh, E. Seeman, J. de Jong, J. van den Bergh, High-resolution in vivo imaging of bone and joints: a window to microarchitecture, *Nat. Rev. Rheumatol.* 10 (5) (2014) 304–313.
- [15] J.A. MacNeil, S.K. Boyd, Accuracy of high-resolution peripheral quantitative computed tomography for measurement of bone quality, *Med. Eng. Phys.* 29 (10) (2007) 1096–1105.
- [16] M.T. Schram, S.J.S. Sep, C.J. van der Kallen, P.C. Dagnelie, A. Koster, N. Schaper, R.M.A. Henry, C.D.A. Stehouwer, The Maastricht Study: an extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities, *Eur. J. Epidemiol.* 29 (6) (2014) 439–451.
- [17] World Health Organization, Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation, 2006 (Geneva, Switzerland).
- [18] WHO's ATC/DDD index 2016 [internet]. Oslo: WHO Collaborating Centre for Drug Statistics Methodology ([updated 2015 Dec 16; cited 2016 Nov 3]. Available from: http://www.whocc.no/atc_ddd_index/.

- [19] J.T. Nielen, J.H. Driessen, P.C. Dagnelie, A. Boonen, B. van den Beemt, H.A. van Onzenoort, et al., Drug utilisation in the Maastricht Study: a comparison with nationwide data, *Forthcoming*, 2016.
- [20] S. Boutroy, M.L. Bouxsein, F. Munoz, P.D. Delmas, In vivo assessment of trabecular bone microarchitecture by high-resolution peripheral quantitative computed tomography, *J. Clin. Endocrinol. Metab.* 90 (12) (2005) 6508–6515.
- [21] S. Khosla, B.L. Riggs, E.J. Atkinson, A.L. Oberg, L.J. McDaniel, M. Holets, et al., Effects of sex and age on bone microstructure at the ultradistal radius: a population-based noninvasive in vivo assessment, *J. Bone Miner. Res.* 21 (1) (2006) 124–131.
- [22] J.B. Pialat, A.J. Burghardt, M. Sode, T.M. Link, S. Majumdar, Visual grading of motion induced image degradation in high resolution peripheral computed tomography: impact of image quality on measures of bone density and micro-architecture, *Bone* 50 (1) (2012) 111–118.
- [23] Y. Pauchard, A.M. Liphardt, H.M. Macdonald, D.A. Hanley, S.K. Boyd, Quality control for bone quality parameters affected by subject motion in high-resolution peripheral quantitative computed tomography, *Bone* 50 (6) (2012) 1304–1310.
- [24] A. Laib, H.J. Hauselmann, P. Rügsegger, In vivo high resolution 3D-QCT of the human forearm, *Technol. Health Care* 6 (5–6) (1998) 329–337.
- [25] A. Laib, P. Rügsegger, Comparison of structure extraction methods for in vivo trabecular bone measurements, *Comput. Med. Imaging Graph.* 23 (2) (1999) 69–74.
- [26] T. Hildebrand, P. Rügsegger, A new method for the model-independent assessment of thickness in three-dimensional images, *J. Microsc.* 185 (1) (1997) 67–75.
- [27] H.R. Buie, G.M. Campbell, R.J. Klinck, J.A. MacNeil, S.K. Boyd, Automatic segmentation of cortical and trabecular compartments based on a dual threshold technique for in vivo micro-CT bone analysis, *Bone* 41 (4) (2007) 505–515.
- [28] A.J. Burghardt, H.R. Buie, A. Laib, S. Majumdar, S.K. Boyd, Reproducibility of direct quantitative measures of cortical bone microarchitecture of the distal radius and tibia by HR-pQCT, *Bone* 47 (3) (2010) 519–528.
- [29] W. Pistoia, B. van Rietbergen, E.M. Lochmüller, C.A. Lill, F. Eckstein, P. Rügsegger, Estimation of distal radius failure load with micro-finite element analysis models based on three-dimensional peripheral quantitative computed tomography images, *Bone* 30 (6) (2002) 842–848.
- [30] N. Dalzell, S. Kaptoge, N. Morris, A. Berthier, B. Koller, L. Braak, B. van Rietbergen, J. Reeve, Bone micro-architecture and determinants of strength in the radius and tibia: age-related changes in a population-based study of normal adults measured with high-resolution pQCT, *Osteoporos. Int.* 20 (10) (2009) 1683–1694.
- [31] S.N. Pramojanee, M. Phimphilai, N. Chattipakorn, S.C. Chattipakorn, Possible role of insulin signaling in osteoblasts, *Endocr. Res.* 39 (4) (2014) 144–151.
- [32] U.K. Prospective Diabetes Study Group, U.K. prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease, *Diabetes* 44 (11) (1995) 1249–58.
- [33] S.Y. Goh, M.E. Cooper, Clinical review: The role of advanced glycation end products in progression and complications of diabetes, *J. Clin. Endocrinol. Metab.* 93 (4) (2008) 1143–1152.
- [34] F. Gregorio, S. Cristallini, F. Santeusano, P. Filippini, P. Fumelli, Osteopenia associated with non-insulin-dependent diabetes mellitus: what are the causes? *Diabetes Res. Clin. Pract.* 23 (1) (1994) 43–54.
- [35] T.J. Beck, T.L. Oreskovic, K.L. Stone, C.B. Ruff, K. Ensrud, M.C. Nevitt, et al., Structural adaptation to changing skeletal load in the progression toward hip fragility: the study of osteoporotic fractures, *J. Bone Miner. Res.* 16 (6) (2001) 1108–1119.
- [36] J. Starup-Linde, S.A. Eriksen, S. Lykkeboe, A. Handberg, P. Vestergaard, Biochemical markers of bone turnover in diabetes patients – a meta-analysis, and a methodological study on the effects of glucose on bone markers, *Osteoporos. Int.* 25 (6) (2014) 1697–1708.
- [37] K. Hygum, J. Starup-Linde, T. Harsløf, P. Vestergaard, B.L. Langdahl, Mechanisms in endocrinology: Diabetes mellitus, a state of low bone turnover – a systematic review and meta-analysis, *Eur. J. Endocrinol.* 176 (3) (2017) R137–R157.
- [38] R. Basu, J. Peterson, R. Rizza, S. Khosla, Effects of physiological variations in circulating insulin levels on bone turnover in humans, *J. Clin. Endocrinol. Metab.* 96 (5) (2011) 1450–1455.
- [39] I. Levinger, E. Seeman, G. Jerums, G.K. McConell, M.S. Rybchyn, S. Cassar, et al., Glucose-loading reduces bone remodeling in women and osteoblast function in vitro, *Phys. Rep.* 4 (3) (2016).
- [40] M. Osima, R. Kral, T.T. Borgen, I.K. Høgestøl, R.M. Joakimsen, E.F. Eriksen, A. Bjørnerem, Women with type 2 diabetes mellitus have lower cortical porosity of the proximal femoral shaft using low-resolution CT than nondiabetic women, and increasing glucose is associated with reduced cortical porosity, *Bone* 97 (2017) 252–260.
- [41] K.S. Button, J.P. Ioannidis, C. Mokrysz, B.A. Nosek, J. Flint, E.S. Robinson, M.R. Munafò, Power failure: why small sample size undermines the reliability of neuroscience, *Nat. Rev. Neurosci.* 14 (5) (2013) 365–376.
- [42] T.P. van Staa, H.G. Leufkens, L. Abenhaim, B. Zhang, C. Cooper, Use of oral corticosteroids and risk of fractures, *J. Bone Miner. Res.* 15 (2000) 993–1000.
- [43] R. Zebaze, E. Seeman, Cortical bone: a challenging geography, *J. Bone Miner. Res.* 30 (1) (2015) 24–29.
- [44] R. Zebaze, A. Ghasem-Zadeh, A. M'bala, E. Seeman, A new method of segmentation of compact-appearing, transitional and trabecular compartments and quantification of cortical porosity from high resolution peripheral quantitative computed tomographic images, *Bone* 54 (2013) 8–20.