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Glucose variability and low bone turnover in people with type 2 diabetes

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ABSTRACT

Introduction: Type 2 diabetes (T2D) is related to an increased fracture risk and low bone turnover. However, the mechanisms are not elucidated. In the present study we investigate the association between glycemic variability and bone turnover markers.

Methods: 100 participants with T2D and 100 age and gender matched controls were included in this cross-sectional study. All participants with T2D were equipped with a continuous glucose monitoring (CGM) sensor for 3 days (CGMS iPro Continuous Glucose Recorder; Medtronic MiniMed). The dawn glucose levels were defined as a morning period starting 1 h before breakfast ending 1 h post ingestion. On all participants serum (s)-C-terminal cross-linked telopeptide of type-I collagen (CTX), s-procollagen type 1 amino terminal propeptide (P1NP), and s-sclerostin were measured.

Results: Participants with T2D displayed significantly lower levels of the bone resorption marker s-CTX and the bone formation marker s-P1NP compared to controls. S-CTX was significantly negatively associated with the mean amplitude of glycemic excursions (MAGE) and the dawn glucose levels whereas s-P1NP only was significantly negatively associated with the dawn glucose levels while it was borderline significantly associated with MAGE ($p = 0.05$). S-CTX and s-P1NP were significantly lower among the 50% with the highest dawn glucose levels compared to the 50% lowest dawn glucose levels also after adjustment for age, gender, glycated hemoglobin A1c (HbA1c), and body mass index (BMI).

Conclusion: We observed that the amplitude of glycemic excursions and rise in dawn glucose was negatively associated with bone turnover markers. Future research is needed to determine whether reduction of the amplitude of glycemic excursions increase bone turnover markers.

1. Introduction

Type 2 diabetes (T2D) is associated with an increased risk of fracture [1]. This may seem paradoxical as bone mineral density is increased in people with T2D compared to controls [2]. However, hyperglycemia is associated with decreased circulating bone turnover markers [3–5] and histomorphometry [4] show decreased bone formation in people with T2D compared to controls. Thus, it has been hypothesized that the increased risk of fracture is due to accumulation of microfractures caused by low bone turnover. Sclerostin, a Wnt pathway inhibitor that inhibits bone formation, is reported to be increased in T2D patients compared to controls [3]. In vitro studies report that hyperglycemia increases the osteocytes production and release of sclerostin and thereby this mechanism may inhibit bone formation and indirectly bone resorption [6,7]. In mice with T2D evidence of a decreased bone formation is found by histomorphometry and in these bone samples the

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osteocytes displayed pro-inflammatory changes compared to non-diabetic mice [8]. Changes in glucose levels may directly or indirectly affect bone turnover. In vitro studies report that hyperglycemia increases the osteocytes production and release of sclerostin and thereby this mechanism may inhibit bone formation and indirectly bone resorption [6,7]. In patients with diabetes, plasma glucose levels are negatively associated with the levels of bone turnover markers [9]. In diabetes, the plasma glucose level may vary during the day and this phenomenon of glycemic variability [10] may influence the level of bone turnover markers; however, the relationship between glycemic variability and bone turnover has not been examined. Glycemic variability and bone turnover marker levels may be influenced by physical activity [11,12], thus we examined both glycemic variability and physical activity in the present study.

In the present cross-sectional study we aimed to investigate bone turnover marker levels in people with T2D compared to controls, and investigate effects of glycemic variability and physical activity on bone turnover markers. The participants with type 2 diabetes had a relatively short diabetes duration, however it has previously been shown that bone turnover marker levels are decreased in newly diagnosed T2D and in the prediabetes state compared to controls [13,14]. We hypothesized that levels of C-terminal cross-linked telopeptide of type-I collagen (CTX) and procollagen type 1 amino terminal propeptide (P1NP) would be decreased whereas sclerostin would be increased in T2D compared to controls. We also investigated that glycemic variability would be related with bone turnover regardless of physical activity.

2. Methods

The details of the study have been described elsewhere [15]. In brief 100 participants with T2D and 100 age and gender matched controls were included. Participants were recruited from outpatient clinics at Aarhus University Hospital and advertising in the local press. Inclusion criteria for T2D patients were diabetes duration less than 5 years and a diabetes diagnosis accordingly to WHO criteria, age $\geq$ 18 years. Controls were excluded if fasting plasma glucose (FPG) or oral glucose tolerance test (OGTT) revealed undiagnosed diabetes. Participants were excluded if there were acute or chronic infections, prior or current malignancy, end-stage renal disease, pregnancy or lactation, and body weight $>$ 120 kg. In total 196 participants were included, four participants were excluded; For two participants there were not enough material for analysis of bone turnover markers and these and their two matched controls were excluded.

2.1. Assessments

All participants with T2D were equipped with a continuous glucose monitoring (CGM) sensor for 3 days (CGMS iPro Continuous Glucose Recorder; Medtronic MiniMed). CGM data from 12 patients were excluded from this study because of use of missing CGM data (less than 24 h of successful CGM recording). The participants were instructed to maintain their usual diet and treatment and were instructed not to change their meal patterns during the period of CGM. The mean amplitude of glycemic excursions (MAGE) was calculated from glycemic data days 2 and 3 to assess individual glycemic variation. MAGE was defined as the average of absolute values of differences between adjacent peaks and nadirs for all differences greater than 1 SD [10]. MAGE is sensitive to large excursions in blood glucose and has previous been proposed as a component to assess glycemic control along with HbA1c, and fasting blood sugar [16]. The dawn glucose levels were defined as a morning period starting 1 h before breakfast ending 1 h post ingestion, that may play a role in glycemic regulation in T2D [17].

An Actiheart accelerometer (Cambridge Neurotechnology Ltd., Cambridge, UK) was worn by both the T2D and participants in the control group for up to 6 days. Acceleration was measured using a piezoelectric element. When a force is applied upon a crystal during acceleration, stress in the crystal generates an electrical charge that is relative to the applied force; this change in charge is then measured. The amount of force is relative to applied acceleration as described by Newton's law of motion. The Actiheart accelerometer has a dynamic range of _25 m/s2 (_2.5 g) and its sensitivity per bit is 0.2 m/s2 (0.02 g). The activity output was measured in counts per min (cpm) and an average was calculated for daytime (08.00–24.00) and night time (24.00–08.00). Data on activity is available for 169 participants.

Clinical data including fasting blood samples taken in the morning were obtained before recording of CGM and Actiheart. The blood samples were separated and the resulting plasma and serum-aliquots were stored at −80 °C until measurements were performed on freshly thawed aliquots by a single analyst, blinded to clinical status. Serum levels of CTX and P1NP were measured on an automated analyzer according to manufacturer instructions. Serum sclerostin was measured in duplets by a sclerostin ELISA assay from Biomedica (Vienna, Austria) according to kit-manufacturer instructions. The inter-assay analytical variation coefficients according to the manufacturers were serum (s)-CTX (<6%), s-P1NP (<4%), and s-sclerostin (<10%).

The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was determined from FPG and fasting plasma insulin (FPI) using the model: $\frac{(FPG\ x\ FPI)}{22.5.}$

2.2. Statistical analysis

Descriptive statistics were used to present participant characteristics and the relation to biochemical bone turnover markers. Normal distribution was assessed visually by Q-Q plots. Mean and standard deviation (SD) is presented when appropriate and otherwise median and interquartile range (IQR). STATA 16 was used to perform the analyses. Unpaired *t*-test or Wilcoxon signed rank-sum test was performed to test for differences as appropriate. Bartletts test was performed to determine if the t-test should be performed with equal or unequal variances. Linear regression adjusted by diabetes status was performed to assess the relation between patient characteristics, glycemic control, activity, and biochemical markers of bone turnover. Multiple linear regression models were based on statistically significant variables in single linear regression ($p < 0.05$), however, only one variable was selected if variables correlated with each other. Assumptions were checked when performing the linear regression models; assumption of normal distribution, assumption of linearity between dependent and independent variable, assumption of reliability, and assumption of homoscedasticity. The multiple linear regressions were divided by gender and diabetes status (yes/no). Use of glucose-lowering drugs were divided into non-users, metformin users, sulphonylureas users, glucagon-like receptor agonist 1 analogue (GLP-1 receptor analogue) users, insulin users, and dipeptidylpeptidase-IV inhibitors (DPP-IVi) users. In the adjusted analysis HOMA-IR was converted to logartitmic scale (log(HOMA-IR)) to ensure normal distribution. Bone turnover markers were compared i) in the 50% least active vs the 50% most active stratified by diabetes status and ii) in the diabetic patients with the 50% largest glycemic variability vs the 50% with the least variability

3. Results

The characteristics of the participants are displayed in Table 1. People with T2D had a larger body mass index (BMI) (30 kg/m^2 ± 4.7 vs. 26 kg/m^2 ± 4.0), higher glycated hemoglobin A1c (HbA1c) (6.5% ± 0.6 vs, 5.6% ± 0.3), and HOMA-IR (1.4, IQR 1.0–2.1 vs. 0.9, IQR 0.6–1.4) and lower activity count during both day (28 cpm ±15 vs. 41 cpm ±19) and night time (2.2 cpm, IQR 1.2–3.6 vs. 2.8 cpm, IQR 1.6–5.0) compared to the controls as previously reported [18]. Among the people with T2D, 36 were treated with metformin, 13 were treated with sulphonylureas, seven were treated with insulin, three were treated with DPP-IV inhibitors, and two were treated with GLP-1 receptor analogues.

Table 1
patient characteristics. Presented as mean ± SD unless otherwise is stated.

Characteristic	Type 2 diabetes (n = 98)	Controls (n = 98)
Age (years)	58 ± 9.7	58 ± 9.7
Gender (n males)	50 (51%)	50 (51%)
Waist hip ratio[a]	0.98 ± 0.07	0.94 ± 0.08
BMI (kg/m^2)[a]	30 ± 4.7	26 ± 4.0
Diabetes duration (years)	2.0 ± 1.4	
eGFR (ml/min)	88 ± 18	82 ± 14
Smoking		
Current smoker (n)	20 (20%)	21 (21%)
Former smoker (n)	36 (37%)	31 (32%)
Never smoker (n)	41 (42%)	46 (47%)
Glycemic markers		
HbA1c (%)[a,b]	6.5 ± 0.6	5.6 ± 0.3
HbA1c (mmol/mol)[a,b]	48 ± 6.6	37 ± 3.3
Plasma fasting glucose (mmol/l) [a,b]	6.8 ± 1.3	5.2 ± 0.6
Median (IQR)fasting insulin levels (pmol/l)[a]	58 (43–95)	42 (27–65)
Median (IQR) HOMA-IR (U)[a]	1.4 (1.0–2.1)	0.9 (0.6–1.4)
Glycemic variability (n = 86)		
Mean (mmol/l)	7.0 ± 1.1	
SD (mmol/l)	1.4 ± 0.6	
MAGE (mmol/l)	3.6 ± 2.0	
Dawn glucose levels (mmol/l)	1.3 ± 0.87	
Activity (n = 169)		
Activity count day 08–24 (counts/min)[a]	28 ± 15	41 ± 19
Median (IQR) Activity night 24–08 (counts/min)[a]	2.2 (1.2–3.6)	2.8 (1.6–5.0)
Bone markers (n = 196)		
CTX (ng/l)[a]	310 ± 170	360 ± 160
P1NP (ng/ml)[a]	40 ± 19	53 ± 23
Sclerostin (pmol/l)	38 ± 15	41 ± 18

[a] Significantly different between people with type 2 diabetes and controls ($p < 0.05$).
[b] Using unequal variances due to Bartletts test.

3.1. Bone markers and association with participant characteristics and glycemic markers

Participants with T2D displayed significantly lower levels of the bone resorption marker s-CTX and the bone formation marker s-P1NP compared to controls. S-sclerostin did not differ between people with T2D and controls. In single linear regression analysis s-CTX was associated with BMI (−8.9 ng/l, 95% CI: −14; −4.1), HbA1c (−39 ng/l, 95% CI: −73;-5.2), FPG (−25 ng/l, 95% CI:-43;-6.0). However, when stratifying the analysis by diabetes status only BMI remained significantly associated with s-CTX among people with T2D (−8.1 ng/l, 95% CI: −15;-1.1). In single linear regression analysis, s-P1NP was associated with BMI (−1.1 ng/ml, 95% CI: −1.7;-0.48), male gender (−8.2 ng/ml, 95% CI: −14;-2.1), (HbA1c (−7.8 ng/ml, 95% CI: −12;-3.4), and FPG (−4.6 ng/ml, 95% CI: −7.1;-2.2), but only BMI remained significantly associated with s-P1NP among people with T2D (−0.84 ng/ml, 95% CI: −1.7;-0.03) and only male gender was associated with s-P1NP in controls (−9.7 ng/ml, 95% CI: −19;-0.70). Fig. 1 illustrates the association between CTX and P1NP and HbA1c for all participants and stratified by diabetes status and shows that the association is abolished when adjusted by diabetes status. Sclerostin was associated with age (0.41 pmol/l, 95% CI: 0.17; 0.65) and estimated glomerular filtration rate (eGFR) (−0.24 pmol/l, 95% CI: −0.39;-0.010) in single linear regression analysis and also when only investigating T2D (0.39 pmol/l, 95%: 0.079;0.70) and − 0.31 pmol/l, 95% CI: −0.48;-0.15, respectively). In controls s-sclerostin was only associated with age (0.43 pmol/l, 95% CI: 0.069; 0.79).

3.2. Bone markers and glycemic variability in T2D

Table 2 presents the single linear regression analysis of bone markers and parameters of glycemic variability. S-CTX was significantly negatively associated with MAGE and the dawn glucose levels whereas s-P1NP only was significantly negatively associated with the dawn glucose levels while it was borderline significantly associated with MAGE ($p = 0.05$). These associations remained when adjusting by log (HOMA-IR). When adjusting for activity count, s-CTX was still significantly associated with dawn glucose levels and borderline associated with MAGE ($p = 0.08$) and s- P1NP was borderline significantly associated with dawn glucose levels ($p = 0.06$) and not associated with MAGE ($p = 0.14$). In multiple adjusted analyses S-CTX was still associated with the dawn glucose levels (−53 ng/l, 95% CI: −98;-7.4) and borderline significantly associated with MAGE (−18 ng/l, 95% CI: −38; 2.7) after adjusting for age, gender, BMI, use of glucose-lowering drugs, and HbA1c and also when additionally adjusting by activity count. S-P1NP was not significantly associated with the dawn glucose levels (−4.5 ng/ml, 95% CI: −9.4;0.3) or MAGE (−1.7 ng/ml, 95% CI: −4.0; 0.66) after adjusting for age, gender, BMI, use of glucose-lowering drugs, and HbA1c in multiple linear regression or when additionally adjusted by activity count. When adjusting for age, gender, log(HOMA-IR), use of glucose-lowering drugs, and HbA1c, s-P1NP and s-CTX were significantly associated with dawn glucose levels (−5.5 ng/ml 95% CI -10;-0.55 and − 61 ng/l 95% CI -107;-16), respectively). A dichotomized model was used to illustrate whether larger differences in MAGE and dawn glucose levels influence the bone turnover marker levels. Fig. 2 displays s-CTX and s-P1NP levels by the 50% with lowest and highest dawn glucose levels and by the 50% with lowest and highest MAGE. Both s-CTX and s-P1NP were significantly lower among the 50% with the highest dawn glucose levels compared to the 50% lowest dawn glucose levels also after adjustment for age, gender, use of glucose-lowering drugs, HbA1c, and BMI. We observed no difference in any bone marker between the 50% with lowest and highest CGM mean and CGM SD. S-sclerostin was not significantly associated with any parameter of glycemic variability.

3.3. Bone markers and activity count

Table 2 presents the single linear regression analysis of bone markers and the activity count in day time. Sclerostin was significantly negatively associated with the activity count, however in analyses stratified by diabetes status this was only present in controls. S-CTX and s-P1NP were significantly positively associated with the activity count in the entire population also when adjusted by diabetes status. Fig. 3 display the levels of s-sclerostin stratified the 50% with lowest (sedentary) and highest (active) activity count during the day and by diabetes status. Among the 50% most active, only 29 were diagnosed with T2D whereas 55 were controls. S-sclerostin levels were not different in patients with diabetes by activity level, however controls displayed lower s-sclerostin levels in those active compared to the more sedentary controls (38 pmol/l, 95% CI: 34;42 vs. 46 pmol/l, 95% CI 38;53).

4. Discussion

To our knowledge, this is the first study to report on bone turnover markers and glycemic variability in people with T2D. As we hypothesized, we found lower levels of s-CTX and s-P1NP in people with T2D compared to controls. Unlike previous studies, s-sclerostin levels did not differ between people with T2D and controls. We observed a negative association between glycemic variability and CTX and P1NP.

We have confirmed previous evidence of low bone turnover markers levels in people with T2D [3]. In the entire cohort we observed that s-CTX and s-P1NP were related to FPG and HbA1c, however this association was abolished by adjustment for diabetes status and not present in controls or people with T2D in stratified analyses, and thus the low bone

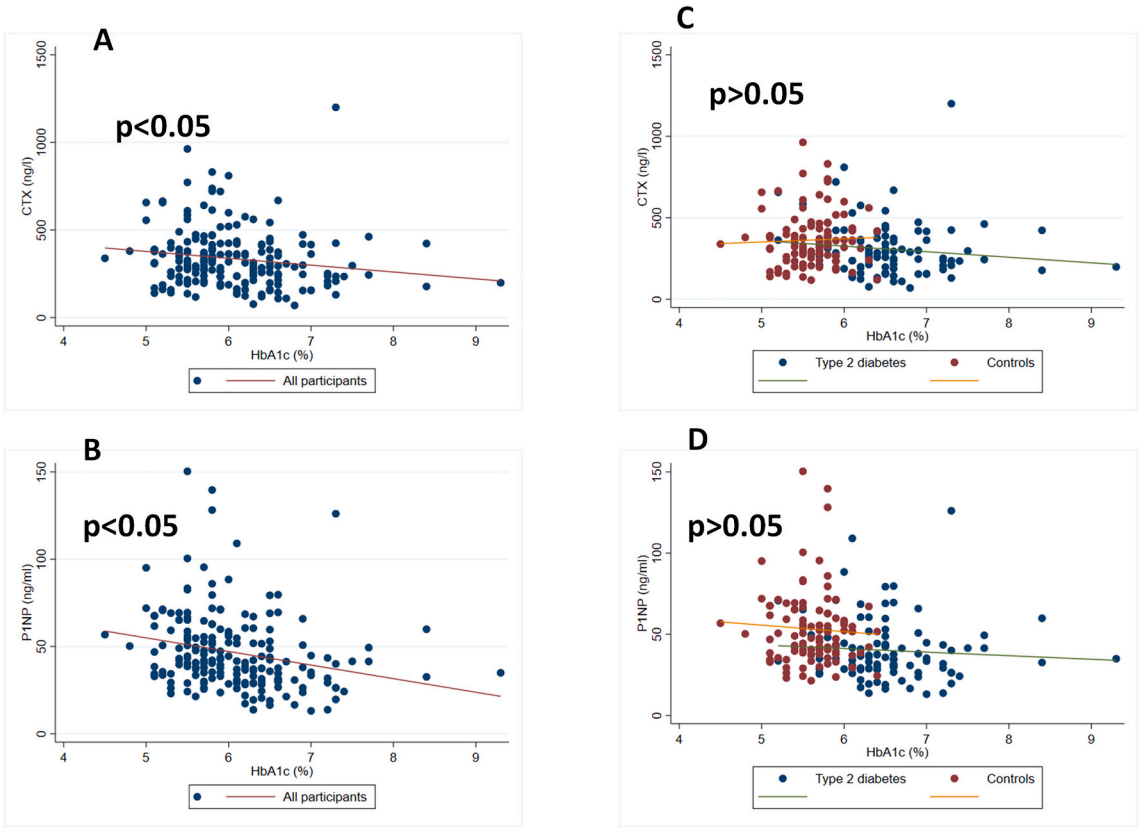

Fig. 1. CTX and P1NP levels scatterplot and regression-line by HbA1c. A) CTX by Hba1c in the entire population, B) P1NP by Hba1c in the entire population, C) CTX by Hba1c stratified by diabetes status, D) P1NP by Hba1c stratified by diabetes status.

Table 2
Associations between bone markers, glycemic variability and activity. Results presented as mean (95% CI).

Glycemic variability	N	CTX ng/l	P1NP ng/ml	Sclerostin pmol/l
Mean (mmol/l)	85	-11 (−46; 24)	−0.75 (−4.8; 3.3)	−1.6 (−4.7; 1.5)
SD (mmol/l)	85	−44 (−100; 16)	−1.3 (−8.3; 5.8)	−1.8 (−7.3; 3.7)
MAGE (mmol/l)	85	−21 (−38; −3.4)[a]	−2.0 (−4.1; 0.02)	0.08 (−1.5; 1.7)
Dawn glucose levels (mmol/l)	86	−59 (−99; −19)[a]	−5.0 (−9.4; −0.63)[a]	−2.8 (−6.6; 0.89)
Activity				
Activity count 08–24 (counts/min)	169	1.9 (0.54; 3.2)[a]	0.29 (0.13; 0.45)[a]	−0.15 (−0.28; −0.02)[a]
Activity count 08–24 (counts/min) in T2D	90	3.2 (1.1; 5.4)[a]	0.28 (0.038; 0.52)[a]	−0.11 (−0.32; 0.11)
Activity count 08–24 (counts/min) in controls	79	0.03 (−1.8; 0.1.9)	0.10 (−0.13; 0.33)	−0.26 (−0.45; −0.080)[a]

[a] Significantly associated ($p < 0.05$).

turnover is related to diabetes status. Among people with T2D, we observed an association with lower s-CTX and s-P1NP levels in those individuals with largest glucose excursions (MAGE) and those with the largest glucose rise during dawn. S-CTX and s-P1NP were measured fasting in the morning and therefore the glucose rise during dawn is temporally related to the bone turnover. The present study shows an association between the amplitude of glucose excursions and bone turnover markers whereas the average glucose level was not associated. T2D patients with poor glycemic control have previously been reported to have lower bone turnover markers [19,20]. In these studies glycemic control was estimated by HbA1c [19,20] and glycemic levels were higher compared to our study. The amplitude of glucose excursions may in the glycemically well controlled T2D in this study reveal bone alterations which may not be detected by HbA1c. This may be by a direct effect of hyperglycemia on osteoclasts and osteoblasts. In vitro studies have shown indices of low bone resorption [21] and bone formation [22] in osteoclasts and osteoblasts, respectively. In patients with T2D, the fracture risk is increased despite of an increased bone mineral density (BMD) [2]. It is hypothesized that the bones in patients with type 2 diabetes hypermineralize and due to low bone turnover accumulate micro fractures which cause clinical fractures [5]. Glucose fluctuations in patients with T2D may contribute to hypermineralization and may per se cause the increased fracture risk as CTX and P1NP have been reported to be negatively associated with BMD [23] and we report that associations between MAGE and dawn glucose levels and CTX and P1NP and hyperglycemia seem to impair bone turnover on the cellular level.

It is shown that addition of glucose does not interfere with the measurement of s-C-terminal cross-linked telopeptide of type-I collagen (CTX) or s-Procollagen type 1 amino terminal propeptide (P1NP) thus assay interference does not explain the low level of bone turnover markers in T2D [24]. Another possible cause for both low bone turnover and increased fracture risk in patients with T2D is the accumulation of advanced glycation end-products (AGEs) which have been suggested to increase fracture risk and reduce CTX levels due to enzymatic alterations of the collagen and reduce P1NP levels due to direct effects on the osteoblasts [25]. However, in this hypothesis the bone resorption is not decreased and the combination of normal to high bone resorption marker levels and low bone formation marker levels does not explain the high BMD levels observed in T2D.

Incretin based therapies are related to a low risk of hypoglycemia as insulin is released during hyperglycemia and thereby reduce glycemic variability. Furthermore the GLP-1 RA also improves satiety which

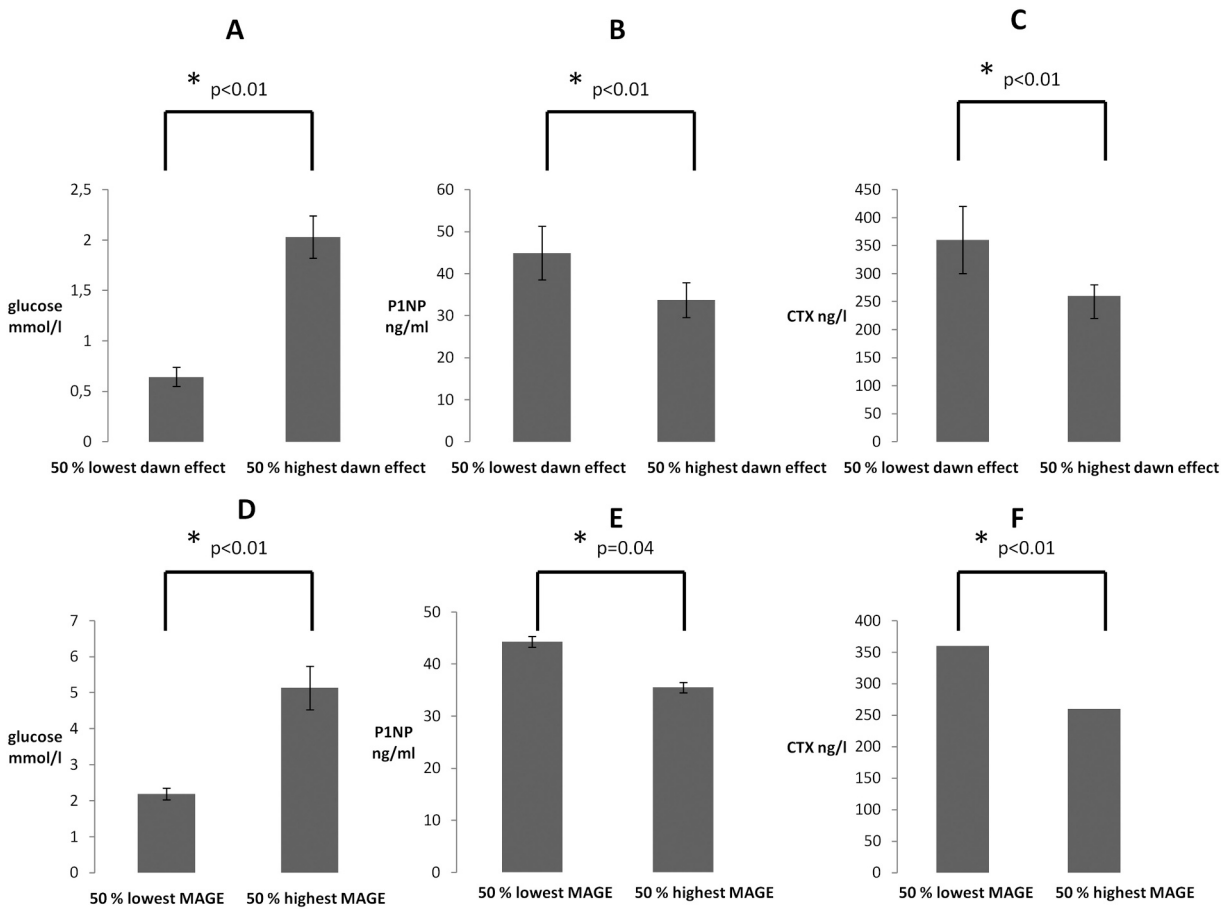

Fig. 2. Levels of CTX and P1NP by 50% highest and lowest dawn glucose levels and 50% highest and lowest MAGE. A) Glucose change by dawn glucose levels, B) P1NP by dawn glucose levels, C) CTX by dawn glucose levels, D) Glucose excursion by MAGE, E) P1NP by MAGE, F) CTX by MAGE.

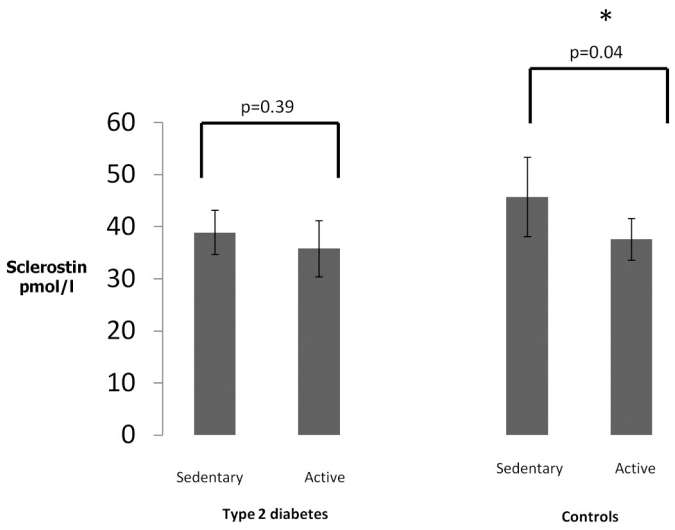

Fig. 3. Levels by activity count and diabetes status. The 50% with the lowest activity count during the day were grouped as sedentary, whereas the 50% with the highest activity count were grouped as active.

reduces postprandial glycemic excursions [26,27]. In clinical trials incretin based therapies have shown reductions in post prandial glycemia and are reported to reduce glycemic variability [27–29]. Incretin hormones may also impact bone turnover and seem to ameliorate glucose excursions. Ingestion of a meal or OGTT decreases s-CTX and s-P1NP acutely whereas no such effect was observed during an isoglycemic intravenous glucose infusion or during somatostatin infusion [30–32]. The gastro-intestinal hormones glucagon-like peptide 2 (GLP-2) and gastric inhibitory peptide (GIP) have been identified to reduce bone resorption. Oral intake of glucagon-like peptide 2 (GLP-2) reduce s-CTX levels and short-term GIP infusion reduced CTX levels in people with T2D [33,34]. Besides a possible direct effect of glucose on bone turnover, this may also be mediated by the incretin hormones. MAGE and dawn glucose levels are also a measure of meal-intake related glucose excursions [35]. Thus, the glycemic excursions measured by MAGE may directly inhibit bone turnover or may be a proxy of an altered gastro-intestinal hormone response. We observed a significant association between MAGE and CTX whereas MAGE and P1NP was only borderline significant associated in the unadjusted analysis. This may be a power issue, however it may also reflect that CTX is a product of bone resorption and P1NP is a product of bone formation and thus it is suggestive that glucose excursions may impair the osteoclasts more than the osteoblasts.

Physical activity is reported to reduce glycemic variability [11] and our findings also indicate that the physical activity count modulate the association between MAGE, dawn glucose levels and bone turnover marker levels as only dawn glucose levels were significantly associated with CTX after adjustment. However, the borderline significant associations observed after adjustment for physical activity may also be due to power issues. Glycemic variability may be a surrogate of insulin resistance and the observed associations may be due to insulin resistance. Furthermore insulin resistance has previously been associated with low bone turnover levels [36], but adjustment by HOMA-IR did not change our results.

In contrast to four previous studies assessing sclerostin levels in T2D and controls, we observed similar sclerostin levels in T2D and controls [37–40]. In the present study, participants with T2D had well-regulated HbA1c of 48 mmol/mol (6.5%) compared to the previous studies in which mean HbA1c were between 53 and 71 mmol/mol (7.0–8.7%) [37–40]. Furthermore, participants with T2D were relatively newly diagnosed with a mean diabetes duration of 2 years whereas it was 10–14 years in three of the previous studies [37,39,40] and 4–5 years in the remaining study [38]. These findings indicate that sclerostin is affected in more severe and long term T2D. Sclerostin is negatively associated with physical activity in postmenopausal women with T2D [41] and sclerostin inhibit bone formation by inhibiting the Wnt pathway [3]. In the present study, sclerostin levels were not related to glucose variability but were associated with physical activity in the controls. In vitro osteocytes cultured from diabetic rats display increasing levels of sclerostin due to hyperglycemia [7] and we speculate glucose levels in the T2D patients were too well-controlled to detect an effect on sclerostin. Still, we observed that sclerostin levels were positively associated with physical activity in controls, whereas in people with T2D the sclerostin response seems blunted. This blunted sclerostin response may be due to a general low physical activity or a hyperglycemic impairment of osteocyte function. In a type 1 diabetes mouse model the response to mechanical loading was reduced in the diabetic mice [42]. Whether this would apply to a T2D model is unknown. Thus the response to mechanical loading may be impaired in diabetes and our findings indicate that the response of the osteocyte is impaired in short term T2D.

The study is limited by its cross-sectional nature and therefore causality cannot be concluded. Our population with T2D was in good glycemic control and effects may be more pronounced in patients with poorer glycemic control. Strengths of the study include the sample size, that CGM was used to capture glycemic variability, that activity was measured objectively.

In conclusion, we observed that the amplitude of glycemic excursions and rise in dawn glucose were negatively associated with bone turnover markers. This finding extends current evidence of glucotoxicity as a mechanism of low bone turnover in T2D. Future research is needed to determine whether reduction of the amplitude of glycemic excursions increase bone turnover markers.

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CRediT authorship contribution statement

JS-L, SL, AH, TKH, PLP, PV, PH, JF, and EL conceptualized and designed the study. JS-L, TKH, PLP, PV, JF, and EL provided funding for the project; JS-L conducted the analyses and drafted the manuscript. JS-L, SL, AH, TKH, PLP, PV, PH, JF, and EL interpreted the data, and revised the manuscript critically. JS-L, SL, AH, TKH, PLP, PV, PH, JF, and EL approved the final version of the manuscript.

References

[1] J. Starup-Linde, M. Frost, P. Vestergaard, B. Abrahamsen, Epidemiology of fractures in diabetes, Calcif. Tissue Int. 100 (2) (2017 Feb) 109–121, https://doi.org/10.1007/s00223-016-0175-x.

[2] 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 Apr) 427–444.

[3] K. Hygum, J. Starup-Linde, T. Harslof, 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 Mar) R137–R157.

[4] J.S. Manavalan, S. Cremers, D.W. Dempster, H. Zhou, E. Dworakowski, A. Kode, et al., Circulating osteogenic precursor cells in type 2 diabetes mellitus, J. Clin. Endocrinol. Metab. 97 (9) (2012) 3240–3250.

[5] K. Hygum, J. Starup-Linde, B.L. Langdahl, Diabetes and bone, Osteoporosis and Sarcopenia 5 (2) (2019 Jun) 29–37, https://doi.org/10.1016/j.afos.2019.05.001.

[6] K.-I. Tanaka, T. Yamaguchi, I. Kanazawa, T. Sugimoto, Effects of high glucose and advanced glycation end products on the expressions of sclerostin and RANKL as well as apoptosis in osteocyte-like MLO-Y4-A2 cells, Biochem. Biophys. Res. Commun. 461 (2) (2015 May 29) 193–199, https://doi.org/10.1016/j.bbrc.2015.02.091.

[7] D.M. Pacicca, T. Brown, D. Watkins, K. Kover, Y. Yan, M. Prideaux, et al., Elevated glucose acts directly on osteocytes to increase sclerostin expression in diabetes, Sci. Rep. 9 (1) (2019 Nov 22) 17353 (-019-52224-3).

[8] B.A. Eckhardt, J.L. Rowsey, B.S. Thicke, D.G. Fraser, K.L. O'Grady, O.P. Bondar, et al., Accelerated osteocyte senescence and skeletal fragility in mice with type 2 diabetes, JCI Insight 5 (9) (2020 May 7), e135236, https://doi.org/10.1172/jci.insight.135236.

[9] J. Starup-Linde, S. Lykkeboe, S. Gregersen, E.M. Hauge, B.L. Langdahl, A. Handberg, et al., Differences in biochemical bone markers by diabetes type and the impact of glucose, Bone 83 (2015 Nov 10) 149–155.

[10] J.R. Rausch, Measures of glycemic variability and links with psychological functioning, Curr Diab Rep 10 (6) (2010 Dec) 415–421.

[11] S.L. Bennetsen, C.S. Feineis, G.E. Legaard, M.P.P. Lyngbæk, K. Karstoft, M. Ried-Larsen, The impact of physical activity on glycemic variability assessed by continuous glucose monitoring in patients with type 2 diabetes mellitus: a systematic review, Front Endocrinol (Lausanne) 11 (2020 Jul 31) 486.

[12] S.C. Manolagas, M. Almeida, Gone with the Wnts: beta-catenin, T-cell factor, forkhead box O, and oxidative stress in age-dependent diseases of bone, lipid, and glucose metabolism, Mol. Endocrinol. 21 (11) (2007 Nov) 2605–2614.

[13] S. Costantini, C. Conte, Bone health in diabetes and prediabetes, World J. Diabetes 10 (8) (2019 Aug 15) 421–445.

[14] H. Li, Y. Wen, P. Liu, L. Zhang, X. Zhang, Y. Liu, et al., Characteristics of bone metabolism in postmenopausal women with newly diagnosed type 2 diabetes mellitus, Clin. Endocrinol. 18 (2021 May).

[15] E. Laugesen, P. Høyem, B. Stausbøl-Grøn, A. Mikkelsen, S. Thrysøe, M. Erlandsen, et al., Carotid-femoral pulse wave velocity is associated with cerebral white matter lesions in type 2 diabetes, Diabetes Care 36 (3) (2013 Mar) 722–728.

[16] L. Monnier, C. Colette, M. Sardinoux, G. Baptista, A. Regnier-Zerbib, D. Owens, Frequency and severity of the dawn phenomenon in type 2 diabetes: relationship to age, Diabetes Care 35 (12) (2012 Dec) 2597–2599.

[17] F. Porcellati, P. Lucidi, G.B. Bolli, C.G. Fanelli, Thirty years of research on the dawn phenomenon: lessons to optimize blood glucose control in diabetes, Diabetes Care 36 (12) (2013 Dec) 3860–3862.

[18] S.L. Cichosz, J. Fleischer, P. Hoeyem, E. Laugesen, P.L. Poulsen, J.S. Christiansen, et al., Objective measurements of activity patterns in people with newly diagnosed type 2 diabetes demonstrate a sedentary lifestyle, Diabet. Med. 30 (9) (2013 Sep) 1063–1066.

[19] N.J. Rianon, S.M. Smith, M. Lee, H. Pervin, P. Musgrave, G.P. Watt, et al., Glycemic control and bone turnover in older Mexican Americans with type 2 diabetes, J. Osteoporos. 2018 (2018 May 13) 7153021.

[20] S.V. Kulkarni, S. Meenatchi, R. Reeta, R. Ramesh, A.R. Srinivasan, C. Lenin, Association of glycemic status with bone turnover markers in type 2 diabetes mellitus, Int J Appl Basic Med Res 7 (4) (2017 Oct-Dec) 247–251.

[21] F. Xu, Y.P. Ye, Y.H. Dong, F.J. Guo, A.M. Chen, S.L. Huang, Inhibitory effects of high glucose/insulin environment on osteoclast formation and resorption in vitro, J. Huazhong Univ. Sci. Technolog. Med. Sci. 33 (2) (2013 Apr) 244–249.

[22] 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 Feb), https://doi.org/10.14814/phy2.12700 (Epub 2016 Feb 4).

[23] J. Starup-Linde, S. Lykkeboe, S. Gregersen, E.M. Hauge, B.L. Langdahl, A. Handberg, et al., Bone structure and predictors of fracture in type 1 and type 2 diabetes, J. Clin. Endocrinol. Metab. 101 (3) (2016 Mar) 928–936.

[24] 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.

[25] K. Asadipooya, E.M. Uy, Advanced glycation end products (AGEs), receptor for AGEs, diabetes, and bone: review of the literature, J Endocr Soc 3 (10) (2019 Jul 10) 1799–1818.

[26] K.B. Degn, C.B. Juhl, J. Sturis, G. Jakobsen, B. Brock, V. Chandramouli, et al., One week's treatment with the long-acting glucagon-like peptide 1 derivative liraglutide (NN2211) markedly improves 24-h glycemia and alpha- and beta-cell function and reduces endogenous glucose release in patients with type 2 diabetes, Diabetes 53 (5) (2004 May) 1187–1194.

[27] H.S. Bajaj, K. Venn, C. Ye, A. Patrick, S. Kalra, H. Khandwala, et al., Lowest glucose variability and hypoglycemia are observed with the combination of a GLP-1 receptor agonist and basal insulin (VARIATION study), Diabetes Care 40 (2) (2017 Feb) 194–200.

[28] A. Rezki, M. Fysekidis, S. Chiheb, E. Vicaut, E. Cosson, P. Valensi, Acute and long-term effects of saxagliptin on post-prandial glycemic response in obese patients with impaired glucose tolerance, Nutr. Metab. Cardiovasc. Dis. 31 (4) (2021 Apr 9) 1257–1266.

[29] C. Irace, R. Fiorentino, C. Carallo, F. Scavelli, A. Gnasso, Exenatide improves glycemic variability assessed by continuous glucose monitoring in subjects with type 2 diabetes, Diabetes Technol. Ther. 13 (12) (2011 Dec) 1261–1263.
[30] A. Bjørnshave, S. Lykkeboe, B. Hartmann, J.J. Holst, K. Hermansen, J. Starup-Linde, Effects of a whey protein pre-meal on bone turnover in participants with and without type 2 diabetes-a post hoc analysis of a randomised, controlled, crossover trial, Diabet. Med. 1 (2020 Dec), e14471.
[31] J.A. Clowes, H.C. Allen, D.M. Prentis, R. Eastell, A. Blumsohn, Octreotide abolishes the acute decrease in bone turnover in response to oral glucose, J. Clin. Endocrinol. Metab. 88 (10) (2003 Oct) 4867–4873.
[32] S. Westberg-Rasmussen, J. Starup-Linde, K. Hermansen, J.J. Holst, B. Hartmann, P. Vestergaard, et al., Differential impact of glucose administered intravenously or orally on bone turnover markers in healthy male subjects, Bone 97 (2017 Jan 23) 261–266.
[33] M.B. Christensen, A.B. Lund, N.R. Jørgensen, J.J. Holst, T. Vilsbøll, F.K. Knop, Glucose-dependent insulinotropic polypeptide (GIP) reduces bone resorption in patients with type 2 diabetes, J Endocr Soc 4 (9) (2020 Jul 16), bvaa097.
[34] D.B. Henriksen, P. Alexandersen, B. Hartmann, C.L. Adrian, I. Byrjalsen, H.G. Bone, et al., Four-month treatment with GLP-2 significantly increases hip BMD: a randomized, placebo-controlled, dose-ranging study in postmenopausal women with low BMD, Bone 45 (5) (2009 Nov) 833–842.
[35] S. Suh, J.H. Kim, Glycemic variability: how do we measure it and why is it important? Diabetes Metab. J. 39 (4) (2015 Aug) 273–282.
[36] R. Fuglsang-Nielsen, E. Rakvaag, P. Vestergaard, B. Hartmann, J.J. Holst, K. Hermansen, et al., Consumption of nutrients and insulin resistance suppress markers of bone turnover in subjects with abdominal obesity, Bone 133 (2020 Apr) 115230.
[37] A. Garcia-Martin, P. Rozas-Moreno, R. Reyes-Garcia, S. Morales-Santana, B. Garcia-Fontana, J.A. Garcia-Salcedo, et al., Circulating levels of sclerostin are increased in patients with type 2 diabetes mellitus, J. Clin. Endocrinol. Metab. 97 (1) (2012 Jan) 234–241.
[38] A.H. van Lierop, N.A. Hamdy, R.W. van der Meer, J.T. Jonker, H.J. Lamb, L. J. Rijzewijk, et al., Distinct effects of pioglitazone and metformin on circulating sclerostin and biochemical markers of bone turnover in men with type 2 diabetes mellitus, Eur. J. Endocrinol. 166 (4) (2012 Apr) 711–716.
[39] A. Gaudio, F. Privitera, K. Battaglia, V. Torrisi, M.H. Sidoti, I. Pulvirenti, et al., Sclerostin levels associated with inhibition of the Wnt/Î²-catenin signaling and reduced bone turnover in type 2 diabetes mellitus, J. Clin. Endocrinol. Metab. 97 (10) (2012) 3744–3750.
[40] M.S. Ardawi, D.H. Akhbar, A. Alshaikh, M.M. Ahmed, M.H. Qari, A.A. Rouzi, et al., Increased serum sclerostin and decreased serum IGF-1 are associated with vertebral fractures among postmenopausal women with type-2 diabetes, Bone 56 (2) (2013 Oct) 355–362.
[41] I. Raska Jr., M. Raskova, V. Zikan, J. Skrha, Prevalence and risk factors of osteoporosis in postmenopausal women with type 2 diabetes mellitus, Cent. Eur. J. Public Health 25 (1) (2017 Mar) 3–10.
[42] A. Parajuli, C. Liu, W. Li, X. Gu, X. Lai, S. Pei, et al., Bone's responses to mechanical loading are impaired in type 1 diabetes, Bone 81 (2015 Dec) 152–160.