

The impact of serum c-peptide levels on bone mineral density in postmenopausal type 2 diabetic women

Berna İmge Aydoğan^{1*}, Adile Begüm Bahçecioğlu Mutlu¹, Çağlar Keskin¹, Funda Seher Özalp Ateş², Sevim Güllü¹

Abstract

Objective: Recent studies suggested that c-peptide had biological activity on bone metabolism. We aimed to evaluate the impact of c-peptide level on bone mineral density (BMD) in postmenopausal type 2 diabetic women.

Material and Methods: Thirty-five insulin naïve type 2 diabetic postmenopausal women were included in our prospective cohort study. Fasting and random c-peptide, parathyroid hormone (PTH), vitamin D, insulin, alkaline phosphatase level (ALP) levels and BMD were evaluated.

Results: The mean age of patients was 61.8±8.56 years. The mean fasting c-peptide, random c-peptide and insulin levels were 3.0±1.24, 7.7±3.7 and 13.9±11.2 µIU/ml, respectively. The mean femoral neck (FN-BMD) and total lumbar spine bone mineral density (L-BMD) were 0.787±0.127 and 0.919±0.122 g/cm², respectively. C-peptide level was associated with total hip BMD (p<0.05) but this relation disappeared after regression analysis adjusted for confounders. A negative correlation between PTH level and FN-BMD was found (p: 0.01). Total hip BMD and L-BMD were negatively correlated with age (p: 0.01 and p: 0.02, respectively). A positive association between DPP4 inhibitor treatment and total hip BMD was observed (p: 0.03).

Conclusions: We observed a positive association between total hip BMD and serum c-peptide level. However, this relationship disappeared in multiple linear regression analysis. Further studies are necessary to evaluate the impact of c-peptide level on the risk of osteoporosis in T2DM.

Keywords: C-peptide, osteoporosis, type 2 diabetes mellitus, bone mineral density

Introduction

Osteoporosis and osteoporotic fractures are associated with increased risk of morbidity and all-cause mortality in elderly population (1-4). Type 2 diabetes mellitus is another health concern which becomes more frequent with ageing and often coexists with osteoporosis in elderly population.

The relationship between type 1 diabetes mellitus (T1DM) and decreased bone mineral density (BMD) is well-known (5). Both T1DM and T2DM are associated with hip fracture risk, whereas the risk is greater in T1DM when compared to T2DM (5-7). However, changes in BMD in T2DM remains controversial (7, 8). A significant increase of BMD in type 2 diabetics was observed in some studies and this finding was attributable to causal relationships between T2DM, hyperinsulinemia, obesity, and protective effect of obesity from osteoporosis (7, 9, 10). Increased insulin levels and insulin resistance were reported to be positively associated with BMD in several studies (11, 12).

These findings were consistent with anabolic action of insulin in bone formation (13). Increased risk of fracture in T2DM despite increased BMD, was suggested to be caused by worsened bone quality (14). Previous studies proposed decreased cortical bone density and low bone material strength index in type 2 diabetic patients (15, 16).

C-peptide level is a reliable index of insulin secretion (17, 18). Although it was previously known as an inactive peptide, recent studies proposed that c-peptide may be involved in physiological pathways (19, 20). A possible role of c-peptide in bone metabolism was suggested in diabetic and non-diabetic patients (21-24). The mechanism in which the c-peptide affects bone mineral density has not been explained clearly. A previous study demonstrated that c-peptide activated ERK1/2 signaling in osteoblast-like cell lines and decreased receptor activator of nuclear factor kappa-B ligand (RANKL) mRNA in vivo (25).

Received 29-07-2019 Accepted 05-09-2019 Available Online 09-09-2019 Published 30-09-2019

¹ Ankara University Faculty of Medicine, Department of Endocrinology and Metabolism Diseases, Ankara, TR

² Ankara University Faculty of Medicine, Department of Biostatistics, Ankara, TR

* Corresponding Author: Berna İmge Aydoğan E-mail: imgehalici@gmail.com Phone: +90 (312) 508 21 00



We aimed to evaluate the impact of serum c-peptide level on bone mineral density in patients with T2DM.

Material and Methods

Patients: Thirty-five postmenopausal women with insulin-naïve Type 2 DM were included in this prospective cross-sectional study. The exclusion criteria of the study were; the use of agents or substances that affect Ca²⁺ homeostasis or metabolism, uncontrolled diabetes (HbA1C level $\geq 8.5\%$), serum creatinine levels ≥ 1.5 mg/dl for men and ≥ 1.4 for women, patients with any kind of malignancy, other conditions that may affect vitamin D/calcium/bone metabolism (Cushing's syndrome, active/chronic liver disease, primary hyperparathyroidism, chronic obstructive pulmonary disease, malabsorption syndromes etc.), fracture history, premature ovarian failure, current/ex-smoking, alcohol consumption and drug abuse. Body mass index was calculated by dividing the weight (in kg) by the square meters of the height. Written informed consents were obtained from all participants. This study was approved by local ethical committee of Ankara University (08-614-19).

Biochemistry: Venous blood samples were obtained after 8 hours of fasting. Fasting blood glucose was measured by using enzymatic method with Roche P800 device. Insulin and c-peptide were measured using the Cobas e411 (Roche Diagnostics, Switzerland). Serum creatinine, calcium, and phosphorus measurements were made with a Beckman Coulter DXI 800 device (Brea, California, USA). Parathyroid hormone (PTH) was measured by the chemiluminescence method with a Beckman Coulter AU5800 device (Brea, California, USA). The 25 hydroxy vitamin D (25OHD) was measured with high performance liquid chromatography method. The corrected calcium (cCa) was calculated by the equation: $cCa = [(4 - \text{albumin}) \times 0.8] + Ca$.

Bone mineral density: Bone mineral density (g/cm²) of femoral neck, total hip and lumbar spine were measured by dual energy X-ray absorptiometry (The Hologic Discovery QDR™ series DXA systems, USA). Bone mineral density was expressed as the amount of mineral (g)/area scanned (cm²). Osteopenia was defined as a BMD between 1.0 and 2.5 SD below that of a "young normal" adult and osteoporosis was defined as femoral or lumbar density of 2.5 standard deviations below that of a young adult according to WHO criteria (26).

Statistical Analysis: All statistical analysis were performed using SPSS version 11.5 (SPSS, Chicago, IL, USA). Kolmogorov-Smirnov test was used to assess the assumption of normality. Normally distributed continuous variables were presented as mean \pm standard deviation. Non-normally distributed continuous variables were presented as median (min-max). Categorical data were summarized as counts and percentages. Nominal variables were assessed with chi-square analysis/Fisher's exact tests. The associations between continuous variables were determined by Pearson/Spearman correlation analysis. For continuous variables, Student's t test/Mann-Whitney U test was used to evaluate the difference between two groups (who had osteoporosis and who had not). A two-sided p-

value < 0.05 was considered as statistically significant. The variables which had a significance level of $p < 0.20$ from the univariate analysis were identified as candidate variables for the multiple linear regression models. The multiple linear regression models was created with Backward Method. A priori power analysis was conducted and showed that at least 29 women should be included to evaluate a relationship between c-peptide level and BMD with 0.5 correlation, using a two-sided hypothesis test with a significance level of 0.05 and 0.8 power.

Results

The mean (\pm s.d) age of patients was 61.8 (± 8.56) years. The patients were postmenopausal for median (min.-max.) 13 (2-28) years. Baseline characteristics and biochemical measurements are summarized in Table 1. The mean total lumbar spine (L-BMD), femoral neck (FN-FMD) and total hip BMD were 0.919 ± 0.122 , 0.787 ± 0.127 , 0.921 ± 0.147 g/cm², respectively.

In the univariate analysis, there was no significant association between osteoporosis and any of the evaluated parameters (Table 2). In adjusted model for age, diabetes duration, menopause duration, nephropathy, PTH and Ca levels; only age was positively associated with presence of osteoporosis ($p: 0.01$, 95% CI=1.05-1.51).

Insulin level was positively correlated with fasting and c-peptide level ($r=0.42$, $p: 0.01$ and $r=0.58$, $p: 0.001$, respectively). Random c-peptide level was negatively correlated with HbA1c level, duration of diabetes, FPG and positively correlated with insulin level ($r=-0.08$, $p<0.001$; $r=-0.22$, $p<0.001$; $r=-0.29$, $p: 0.01$, $r=0.42$, $p<0.001$, respectively) (Table 3).

Lumbar spine total BMD was higher in patients without nephropathy when compared to with nephropathy (0.930 ± 0.117 vs 0.737 ± 0.048 , $p: 0.02$). Associations between L-BMD and biochemical parameters are summarized in Table 4.

Total hip BMD was positively associated with age and fasting c-peptide level ($p: 0.01$ and $p: 0.04$, respectively) (Table 4). Total hip BMD was higher in patients under DPP4 inhibitor treatment (1.021 ± 0.120 vs 0.892 ± 0.143 , $p: 0.02$). Other clinical and biochemical parameters were not associated with total hip BMD. Associations between total hip BMD and biochemical parameters are summarized in Table 4.

Femoral neck BMD was positively associated with age ($p: 0.006$) and negatively associated with PTH level ($p: 0.01$). Associations between FN-BMD and biochemical parameters are summarized in Table 4.

Age was the only parameter associated with L-BMD in multiple linear regression analysis ($p: 0.02$). Parathyroid hormone level was slightly associated with FN-BMD but this relationship was not statistically significant ($p: 0.06$). Total hip BMD was negatively associated with age and positively associated with DPP-4 inhibitor usage ($p: 0.01$ and $p: 0.03$, respectively). Multiple linear regression analyses are summarized in table 5.

Table 1. Baseline characteristics of study participants

Parameters	Mean (\pm s.d) / Median (min.-max.)
Age (years) *	61.8 \pm 8.5
Age of menopause (years) **	46 (43-55)
Menopause duration (years) **	13 (2-28)
Duration of diabetes (years) *	10.5 \pm 7.9
BMI (kg/m ²) *	31.5 \pm 4.4
Waist circumference (cm) *	107.1 \pm 12.9
Hypertension, n(%) (A/P)	25(71.4)/10(28.6)
HbA1C (%) *	7.1 \pm 0.87
FBG (70-100 mg/dl) **	122 (73-216)
Insulin (4-16 uIU/ml) **	12.1 (3.8-71)
Fasting c-peptide (RR:1.1-4.4 ng/ml) *	3.0 \pm 1.2
Random c-peptide *	7.7 \pm 3.8
cCalcium (RR:8.5-10.5 mg/dL) *	9.6 \pm 0.52
Vitamin D (RR:20-100 ng/mL) **	12.1 (5.1-35)
PTH (RR:14-72 pg/mL) **	56 (26.8-160)
TSH (RR:0.5-5.5 mIU/L) **	1.88 (1-6)
ALP (RR: 30-130 U/L)	75.4 \pm 24.5
Medications;	
Metformin, n(%) (A/P)	0(0.0)/35(100.0)
DPP4 inhibitors, n(%) (A/P)	27(77.1)/8(22.9)
Sulphonylurea, n(%) (A/P)	22(62.9)/13(37.1)
Diabetic retinopathy , n(%) (A/P)	32(91.4)/3(8.6)
Diabetic neuropathy, n(%) (A/P)	29(82.9)/6(17.1)
Diabetic nephropathy, n(%) (A/P)	33(94.3)/2(5.7)
Coronary artery disease n(%)	28(80.0)/7(20.0)

*: mean \pm standard deviation, **: median (min.-max.) ,A/P=absent/present, ALP:alkaline phosphatase; BMI; body mass index; cCalcium:corrected calcium; DPP4: dipeptidyl peptidase-4; FPG : fasting plasma glucose; PTH: parathormone; HbA1c: Glycosylated haemoglobin; RR=reference range, SU: sulphonylurea, TSH: thyroid stimulating hormone.

Table 2. Impact of clinical and biochemical parameters on osteoporosis

Parameters	Osteoporosis		p-value
	Absent (n=25)	Present (n=10)	
	Mean (\pm s.d) / Median (min.-max.)		
Age (years)	60.2 \pm 8.4	65.8 \pm 7.9	0.08
Age of menopause (years) **	46 (43-55)	48 (45-53)	0.46
Menopause duration (years)**	10 (2-25)	15.5 (5-28)	0.19
Duration of diabetes (years)*	11.1 \pm 8.6	8.6 \pm 6.1	0.17
BMI (kg/m ²)*	31.76 \pm 4.45	30.75 \pm 4.43	0.54
Waist circumference (cm)*	106.4 \pm 13.4	109.1 \pm 12.1	0.56
HbA1C (%)*	7.2 \pm 0.9	6.7 \pm 0.73	0.25
FBG (mg/dl) **	123 (73-216)	121.5 (76-206)	0.92
Insulin (μ IU/mL)*	15.2 \pm 12.8	10.7 \pm 3.97	0.37
C-peptide (fasting) (ng/ml)*	2.8 \pm 1.2	3.4 \pm 1.4	0.18
C-peptide (random)(ng/ml)**	7.17 (2.1-14.9)	6.4 (3.5-20.9)	0.89
cCalcium (mg/dl)*	9.48 \pm 0.51	9.79 \pm 0.52	0.12
Vitamin D (ng/ml)**	9.9 (5.1-35)	13.7 (5.1-31)	0.36
Parathormon (pg/mL)**	55.4 (26-113)	62.7 (42-160)	0.15
ALP (U/L)*	73.8 \pm 22.8	78.9 \pm 29.1	0.63
TSH (mIU/L)**	2 (1-6)	2 (1-5)	0.92
Hypertension, n(%) (A/P)	7(28.0)/18(72.0)	3 (30.0)/7 (70.0)	0.61
Hyperlipidemia, n(%) (A/P)	4(16.0)/21(84.0)	4 (40.0)/6 (60.0)	0.14
Medications;			
Metformin, n(%) (A/P)	0(0.0)/25(100.0)	0 (0.0)/10 (100.0)	0.28
DPP4 inhibitors, n(%) (A/P)	19(76.0)/6(24.0)	8 (80.0)/2 (20.0)	0.58
Sulphonylurea, n(%) (A/P)	14(56.0)/11(44.0)	8(80.0)/2(20.0)	0.21
Diabetic retinopathy, n(%) (A/P)	23(92.0)/2(8.00)	9(90.0)/1(10.0)	0.28
Diabetic neuropathy, n(%) (A/P)	22(88.0)/3(12.0)	7(70.0)/3(30.0)	0.32
Diabetic nephropathy, n(%) (A/P)	25(100.0)/0(0.0)	8(80.0)/2(20.0)	0.07

** : median (min.-max.), A/P=absent/present, ALP:alkaline phosphatase; BMI; body mass index; cCalcium:corrected calcium; DPP4: dipeptidyl peptidase-4; FPG : fasting plasma glucose; HbA1c: Glycosylated haemoglobin; RR=reference range, SU: sulphonylurea,

Table 3. Associations between fasting/ random c-peptide levels, clinical and biochemical parameters.

Parameters	Fasting c-peptide		Random c-peptide	
	Correlation Coefficient (r)	p-value	Correlation Coefficient (r)	p-value
Age	0.18	0.31	0.11	0.54
Age at menopause	-0.21	0.25	-0.11	0.53
Duration of menopause	0.12	0.51	0.01	0.95
Duration of diabetes	-0.22	0.21	-0.52	0.001
BMI	0.27	0.11	0.24	0.16
Waist circumference	0.11	0.54	0.078	0.65
calcium	0.30	0.08	-0.04	0.79
phosphorus	-0.21	0.22	-0.52	0.02
ALP	0.22	0.22	0.17	0.43
PTH	0.08	0.64	0.29	0.11
Vitamin D	-0.14	0.43	-0.06	0.72
FPG	-0.29	0.09	-0.41	0.01
Insulin	0.42	0.01	0.58	0.001
HbA1c	-0.08	0.64	-0.59	0.001
TSH	-0.22	0.21	-0.28	0.2
Hypertension	0.04	0.79	0.19	0.26
Metformin	-0.08	0.63	0.02	0.91
DPP4 inhibitors	-0.31	0.07	-0.38	0.02
SU	0.03	0.86	-0.31	0.07
Retinopathy	-0.24	0.17	-0.19	0.27
Neuropathy	-0.10	0.54	0.06	0.72
Nephropathy	-0.11	0.55	-0.15	0.38

ALP:alkaline phosphatase; BMI; body mass index; cCalcium:corrected Calcium; DPP4: dipeptidyl peptidase-4; FPG : fasting plasma glucose; PTH: parathormone; HbA1c: Glycosylated haemoglobin; SU: sulphonylurea, TSH: thyroid stimulating hormone.

Table 4. Associations between femoral neck, total hip, lumbar spine BMD, clinical and biochemical parameters.

Parameters	FN-BMD (g/cm ²)		Total hip BMD (g/cm ²)		L-BMD (g/cm ²)	
	r	p-value	r	p-value	r	p-value
Age	-0.45	0.006	-0.42	0.01	0.28	0.08
Age at menopause	-0.07	0.6	0.06	0.72	0.22	0.90
Duration of menopause	-0.28	0.11	-0.21	0.23	0.19	0.20
Duration of diabetes	0.13	0.43	0.25	0.13	0.17	0.32
BMI	0.02	0.88	0.12	0.48	0.14	0.40
Waist circumference	-0.01	0.91	0.16	0.33	0.02	0.87
cCalcium	0.09	0.58	-0.21	0.27	-0.01	0.91
Phosphorus	0.17	0.31	0.03	0.84	0.19	0.25
ALP	0.18	0.30	0.10	0.56	0.05	0.76
PTH	-0.44	0.01	-0.11	0.53	-0.19	0.28
Vitamin D	-0.05	0.74	0.06	0.71	0.004	0.98
FPG	-0.05	0.74	-0.04	0.79	-0.14	0.39
Insulin	-0.20	0.26	0.20	0.25	0.10	0.56
HbA1c	0.17	0.33	0.04	0.78	0.21	0.24
c-peptide (fasting)	-0.13	0.44	-0.34	0.04	-0.33	0.85
c-peptide (random)	-0.27	0.12	-0.06	0.72	-0.14	0.40
TSH	0.10	0.54	0.03	0.85	-0.07	0.56

-r: Correlation Coefficient

Table 5. Multiple linear regression analysis of parameters related to lumbar spine BMD, femur shaft BMD and total hip BMD

Variables	Unstandardized coefficients		Standardized coefficients	t	p-value
	B	Std. error			
L-BMD*	Age	-0.007	0.002	-0.51	0.02
FN-BMD**	PTH	-0.001	0.001	-0.30	0.06
Total hip BMD***	Age	-0.007	0.003	-0.41	0.01
	DPP4 inh.	0.12	0.056	0.34	0.03

*excluded variables: duration of menopause, nephropathy, sulphonylurea treatments. ** excluded variables: Age, random c-peptide level, duration of menopause, sulphonylurea treatment. *** excluded variables: Fasting c-peptide level.

Discussion

In the present study, serum c-peptide level was positively related with total hip BMD in postmenopausal women with diabetes. However, in multivariate analysis we did not observe a significant relationship between serum c-peptide levels and BMD at any sites. Total hip BMD and lumbar spine BMD (L-BMD) were both negatively associated with age. Femoral neck BMD (FN-BMD) was negatively associated with parathyroid hormone (PTH) level. Dipeptidyl peptidase-4 inhibitor treatment was associated with higher total hip BMD.

Previous studies suggested that c-peptide had multiple effects in physiological pathways. Its role in prevention of diabetic vascular complications was demonstrated in several studies (27-29). Regarding to bone metabolism, c-peptide was shown to cause stimulation of Na-K-ATPase and activate Ca²⁺ dependent signaling pathways which are essential for osteoblast activities (20, 30, 31). Stimulation of ERK1/2 by c-peptide via phosphoinositide 3-kinase pathway (PI3K) was demonstrated (32). In osteoblast-like cell lines, activation of ERK1/2 was shown to decrease RANKL mRNA levels (33). In the study of Russo et al., c-peptide prevented the reduction of type I collagen mRNA/protein, in addition to activating of ERK1/2 signaling and reducing RANKL mRNA/protein levels in Saos-2 cells which represent a model for osteoblastic differentiation in human cells (25).

The association between BMD and c-peptide level was investigated in a limited number of clinical studies (21, 23, 24, 34). Results were conflicting in both diabetic and non-diabetic populations (21, 23, 34). A cross-sectional survey was conducted with 6625 participants of National Health and Nutrition Examination Survey. The study showed that there was a significant negative association between serum c-peptide level, total and most regional BMDs in both genders independent of confounding factors (24). However, this relationship was not observed among subjects older than 60 years, whose c-peptide levels were higher when compared to younger (24). A study by Montalcini et al. reported that serum c-peptide was strongly associated with L-BMD in postmenopausal non-diabetic women independent of confounders like insulin, 25-OH-D, PTH, FGF-23 levels and BMI values (23). A recent study reported that serum c-peptide level was inversely associated with fracture risk and positively with BMD, in postmenopausal women without diabetes (35). An alternative hypothesis of this study was that c-peptide may be a marker of osteoporosis rather than a cause (35). In type 1 diabetic patients, glucagon stimulated c-peptide level was shown to be related with lumbar and femoral BMD (21). However, a recent study investigated BMD in type 1 diabetic and non-diabetic premenopausal women, and did not demonstrate its association with urinary c-peptide level (34).

In postmenopausal patients with T2DM, c-peptide level was positively associated with FN-BMD in both genders and inversely with fracture risk in women (22). Authors attributed these findings to anabolic effects of insulin; stimulation of bone formation and osteoblast proliferation.

However, the impact of urinary c-peptide on fracture risk was independent of BMD in women. Authors pointed out a possible favorable effect of c-peptide on bone quality which was not reflected by BMD. In our cohort, FN-BMD was not associated with BMD. In univariate analysis, BMD was positively associated with c-peptide level, but this relationship disappeared after adjustments for confounding factors.

Substrates of DPP4 and their receptors are expressed in bone (36, 37). Glucose dependent insulinotropic peptide (GIP) administration was shown to increase bone density in rats. Glucagon-like peptide 1 receptor agonists were shown to decrease bone loss and increase bone formation in vitro and in vivo (38, 39). A previous meta-analysis suggested that DPP-4 inhibitors was related with reduced fracture risk (40). In our study, DPP4 inhibitor treatment was positively associated with the total hip BMD.

A major strength of our study was the exclusion of many factors that promote to osteoporosis, just as alcohol consumption, current-ex smoking, medications which effect calcium and bone metabolism, comorbidities related to osteoporosis. The major limitation of study was relatively small sample size.

Conclusion

We observed a positive association between total hip BMD and serum c-peptide level. However, this relationship disappeared after adjustment for confounders. A possible link between BMD of total hip may be masked by limited number of participants. Direct effect of c-peptide levels on BMD and fracture risk independent of BMD should be evaluated.

Acknowledgements: None

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: BİA, ABBM, ÇK, SG; Research concept and design, Patient examinations, Research the literature, preparation of the article. Chemical Analysis. FSÖA: Statistical analyzes BİA; Revision of the article.

References

1. Nevitt MC, Ettinger B, Black DM, Stone K, Jamal SA, Ensrud K, et al. The association of radiographically detected vertebral fractures with back pain and function: a prospective study. *Annals of internal medicine*. 1998 May 15;128(10):793-800. PubMed PMID: 9599190.
2. Gold DT. The nonskeletal consequences of osteoporotic fractures. Psychologic and social outcomes. *Rheumatic diseases clinics of North America*. 2001 Feb;27(1):255-62. PubMed PMID: 11285999.
3. Cooper C, Atkinson EJ, Jacobsen SJ, O'Fallon WM, Melton LJ, 3rd. Population-based study of survival after osteoporotic fractures. *American journal of epidemiology*. 1993 May 1;137(9):1001-5. PubMed PMID: 8317445.
4. Magaziner J, Lydick E, Hawkes W, Fox KM, Zimmerman SI, Epstein RS, et al. Excess mortality attributable to hip fracture in white women aged 70 years and older. *American journal of public health*. 1997 Oct;87(10):1630-6. PubMed PMID: 9357344. Pubmed Central PMCID: 1381125.

5. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes--a meta-analysis. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*. 2007 Apr;18(4):427-44. PubMed PMID: 17068657.
6. Janghorbani M, Feskanich D, Willett WC, Hu F. Prospective study of diabetes and risk of hip fracture: the Nurses' Health Study. *Diabetes care*. 2006 Jul;29(7):1573-8. PubMed PMID: 16801581.
7. Bonds DE, Larson JC, Schwartz AV, Strotmeyer ES, Robbins J, Rodriguez BL, et al. Risk of fracture in women with type 2 diabetes: the Women's Health Initiative Observational Study. *The Journal of clinical endocrinology and metabolism*. 2006 Sep;91(9):3404-10. PubMed PMID: 16804043.
8. Chen FP, Kuo SF, Lin YC, Fan CM, Chen JF. Status of bone strength and factors associated with vertebral fracture in postmenopausal women with type 2 diabetes. *Menopause*. 2018 Aug 20. PubMed PMID: 30130285.
9. Rakic V, Davis WA, Chubb SA, Islam FM, Prince RL, Davis TM. Bone mineral density and its determinants in diabetes: the Fremantle Diabetes Study. *Diabetologia*. 2006 May;49(5):863-71. PubMed PMID: 16518589.
10. Reid IR, Evans MC, Cooper GJ, Ames RW, Stapleton J. Circulating insulin levels are related to bone density in normal postmenopausal women. *The American journal of physiology*. 1993 Oct;265(4 Pt 1):E655-9. PubMed PMID: 8238341.
11. Shanbhogue VV, Finkelstein JS, Bouxsein ML, Yu EW. Association Between Insulin Resistance and Bone Structure in Nondiabetic Postmenopausal Women. *The Journal of clinical endocrinology and metabolism*. 2016 Aug;101(8):3114-22. PubMed PMID: 27243136. Pubmed Central PMCID: 4971339.
12. Abrahamsen B, Roholte A, Henriksen JE, Beck-Nielsen H. Correlations between insulin sensitivity and bone mineral density in non-diabetic men. *Diabetic medicine : a journal of the British Diabetic Association*. 2000 Feb;17(2):124-9. PubMed PMID: 10746482.
13. Thrall KM, Lumpkin CK, Jr., Bunn RC, Kemp SF, Fowlkes JL. Is insulin an anabolic agent in bone? Dissecting the diabetic bone for clues. *American journal of physiology Endocrinology and metabolism*. 2005 Nov;289(5):E735-45. PubMed PMID: 16215165. Pubmed Central PMCID: 2387001.
14. Roy B. Biomolecular basis of the role of diabetes mellitus in osteoporosis and bone fractures. *World journal of diabetes*. 2013 Aug 15;4(4):101-13. PubMed PMID: 23961320. Pubmed Central PMCID: 3746082.
15. Ho-Pham LT, Chau PMN, Do AT, Nguyen HC, Nguyen TV. Type 2 diabetes is associated with higher trabecular bone density but lower cortical bone density: the Vietnam Osteoporosis Study. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*. 2018 Sep;29(9):2059-67. PubMed PMID: 29967929.
16. Nilsson AG, Sundh D, Johansson L, Nilsson M, Mellstrom D, Rudang R, et al. Type 2 Diabetes Mellitus Is Associated With Better Bone Microarchitecture But Lower Bone Material Strength and Poorer Physical Function in Elderly Women: A Population-Based Study. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2017 May;32(5):1062-71. PubMed PMID: 27943408.
17. Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes*. 1992 Mar;41(3):368-77. PubMed PMID: 1551497.
18. Ciresi A, Ciccio F, Radellini S, Giordano C. Utility of C-peptide for a reliable estimate of insulin secretion in children with growth hormone deficiency. *Growth hormone & IGF research : official journal of the Growth Hormone Research Society and the International IGF Research Society*. 2016 Aug;29:71-7. PubMed PMID: 27235725.
19. Al-Rasheed NM, Meakin F, Royal EL, Lewington AJ, Brown J, Willars GB, et al. Potent activation of multiple signalling pathways by C-peptide in opossum kidney proximal tubular cells. *Diabetologia*. 2004 Jun;47(6):987-97. PubMed PMID: 15164168.
20. Wahren J, Ekberg K, Johansson J, Henriksson M, Pramanik A, Johansson BL, et al. Role of C-peptide in human physiology. *American journal of physiology Endocrinology and metabolism*. 2000 May;278(5):E759-68. PubMed PMID: 10780930.
21. Lopez-Ibarra PJ, Pastor MM, Escobar-Jimenez F, Pardo MD, Gonzalez AG, Luna JD, et al. Bone mineral density at time of clinical diagnosis of adult-onset type 1 diabetes mellitus. *Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists*. 2001 Sep-Oct;7(5):346-51. PubMed PMID: 11585369.
22. Yamaguchi T, Kanazawa I, Yamamoto M, Kurioka S, Yamauchi M, Yano S, et al. Associations between components of the metabolic syndrome versus bone mineral density and vertebral fractures in patients with type 2 diabetes. *Bone*. 2009 Aug;45(2):174-9. PubMed PMID: 19446053.
23. Montalcini T, Gallotti P, Coppola A, Zambianchi V, Fodaro M, Galliera E, et al. Association between low C-peptide and low lumbar bone mineral density in postmenopausal women without diabetes. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*. 2015 May;26(5):1639-46. PubMed PMID: 25616509. Pubmed Central PMCID: 4428888.
24. Li Y, Liu H, Sato Y. The association between the serum C-peptide level and bone mineral density. *PloS one*. 2013;8(12):e83107. PubMed PMID: 24358252. Pubmed Central PMCID: 3865098.
25. Russo C, Lazzaro V, Gazzaruso C, Maurotti S, Ferro Y, Pingitore P, et al. Proinsulin C-peptide modulates the expression of ERK1/2, type I collagen and RANKL in human osteoblast-like cells (Saos-2). *Molecular and cellular endocrinology*. 2017 Feb 15;442:134-41. PubMed PMID: 28007656.
26. Kanis JA. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: synopsis of a WHO report. WHO Study Group. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*. 1994 Nov;4(6):368-81. PubMed PMID: 7696835.
27. Wahren J, Kallas A, Sima AA. The clinical potential of C-peptide replacement in type 1 diabetes. *Diabetes*. 2012 Apr;61(4):761-72. PubMed PMID: 22442295. Pubmed Central PMCID: 3314360.
28. Gubitosi-Klug RA, Group DER. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: summary and future directions. *Diabetes care*. 2014;37(1):44-9. PubMed PMID: 24356597. Pubmed Central PMCID: 3867991.
29. Lachin JM, McGee P, Palmer JP, Group DER. Impact of C-peptide preservation on metabolic and clinical outcomes in the Diabetes Control and Complications Trial. *Diabetes*. 2014 Feb;63(2):739-48. PubMed PMID: 24089509. Pubmed Central PMCID: 3900540.
30. Francis MJ, Lees RL, Trujillo E, Martin-Vasallo P, Heersche JN, Mobasher A. ATPase pumps in osteoclasts and osteoblasts. *The international journal of biochemistry & cell biology*. 2002 May;34(5):459-76. PubMed PMID: 11906818.

31. Ohtomo Y, Bergman T, Johansson BL, Jornvall H, Wahren J. Differential effects of proinsulin C-peptide fragments on Na⁺, K⁺-ATPase activity of renal tubule segments. *Diabetologia*. 1998 Mar;41(3):287-91. PubMed PMID: 9541168.
32. Kitamura T, Kimura K, Jung BD, Makondo K, Okamoto S, Canas X, et al. Proinsulin C-peptide rapidly stimulates mitogen-activated protein kinases in Swiss 3T3 fibroblasts: requirement of protein kinase C, phosphoinositide 3-kinase and pertussis toxin-sensitive G-protein. *The Biochemical journal*. 2001 Apr 1;355(Pt 1):123-9. PubMed PMID: 11256956. Pubmed Central PMCID: 1221719.
33. Saito M, Marumo K. Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA*. 2010 Feb;21(2):195-214. PubMed PMID: 19760059.
34. Kujath AS, Quinn L, Elliott ME, LeCaire TJ, Binkley N, Molino AR, et al. Different health behaviours and clinical factors associated with bone mineral density and bone turnover in premenopausal women with and without type 1 diabetes. *Diabetes/metabolism research and reviews*. 2015 May;31(4):421-32. PubMed PMID: 25470722. Pubmed Central PMCID: 4406845.
35. Ferro Y, Russo C, Russo D, Gazzaruso C, Coppola A, Gallotti P, et al. Association between low C-peptide and fragility fractures in postmenopausal women without diabetes. *Journal of endocrinological investigation*. 2017 Oct;40(10):1091-8. PubMed PMID: 28401528.
36. Nuche-Berenguer B, Portal-Nunez S, Moreno P, Gonzalez N, Acitores A, Lopez-Herradon A, et al. Presence of a functional receptor for GLP-1 in osteoblastic cells, independent of the cAMP-linked GLP-1 receptor. *Journal of cellular physiology*. 2010 Nov;225(2):585-92. PubMed PMID: 20506394.
37. Zhong Q, Itokawa T, Sridhar S, Ding KH, Xie D, Kang B, et al. Effects of glucose-dependent insulintropic peptide on osteoclast function. *American journal of physiology Endocrinology and metabolism*. 2007 Feb;292(2):E543-8. PubMed PMID: 17003233.
38. Lu N, Sun H, Yu J, Wang X, Liu D, Zhao L, et al. Glucagon-like peptide-1 receptor agonist Liraglutide has anabolic bone effects in ovariectomized rats without diabetes. *PloS one*. 2015;10(7):e0132744. PubMed PMID: 26177280. Pubmed Central PMCID: 4503456.
39. Iepsen EW, Lundgren JR, Hartmann B, Pedersen O, Hansen T, Jorgensen NR, et al. GLP-1 Receptor Agonist Treatment Increases Bone Formation and Prevents Bone Loss in Weight-Reduced Obese Women. *The Journal of clinical endocrinology and metabolism*. 2015 Aug;100(8):2909-17. PubMed PMID: 26043228.
40. Monami M, Dicembrini I, Antenore A, Mannucci E. Dipeptidyl peptidase-4 inhibitors and bone fractures: a meta-analysis of randomized clinical trials. *Diabetes care*. 2011 Nov;34(11):2474-6. PubMed PMID: 22025784. Pubmed Central PMCID: 3198283.