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Circulating Proneurotensin Levels Predict Impaired Bone Mineralisation in Postmenopausal Women With Type 2 Diabetes Mellitus

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ABSTRACT

Context: The mechanisms underlying bone fragility and increased fracture risk observed in individuals with type 2 diabetes (T2D) are not yet fully elucidated. Previous research has suggested a role for neuropeptides in regulating bone metabolism; however, the contribution of the neuropeptide Neurotensin (NT), which is thoroughly implicated in T2D and cardiovascular disease, has not been investigated in this context.

Objective: To study the relationship between circulating levels of the NT precursor proneurotensin (proNT) and bone mineralisation in T2D women.

Materials and Methods: This is a cross-sectional investigation with a longitudinal prospective phase, involving 126 women with T2D who underwent bone density scans and had proNT levels measured. Biomarkers of bone metabolism and inflammation were also assessed. Data on bone mineral density (BMD) after 12 months were available for 49 patients.

Main Outcome Measure: Plasma proNT levels in relation to BMD.

Results: 32% of the participants had osteopenia/osteoporosis and exhibited higher proNT than those with normal BMD (200.8 ± 113.7 vs. 161.6 ± 108.8 pg/mL; $p = 0.013$). ProNT inversely correlated with femur BMD and T-score ($p < 0.01$) and was associated with degraded bone architecture (TBS, $p = 0.02$), and higher OPN, P1NP, TNF-α and IL-1β levels. Baseline proNT correlated with further BMD reduction at the 12-month follow-up, independently of potential confounders ($p = 0.02$).

Conclusions: In women with T2D, greater proNT levels are associated with impaired bone mineralisation and predict mineral density decline overtime. ProNT could potentially serve as a diagnostic tool for identifying patients at higher risk of osteopenia/osteoporosis, suggesting a significant connection between this neuropeptide and bone metabolism in diabetes.

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1 | Introduction

Type 2 diabetes and osteoporosis, both highly prevalent chronic conditions, pose an escalating health burden worldwide. In 2015, 415 million adults had type 2 diabetes diagnosis (8.8% of the global population), with an estimated prevalence growing over 10% by 2040 [1]. Osteoporosis has a prevalence of 23% in women [2], and bone fragility exposes to even greater fracture risk in the presence of diabetes, with unfavourable short- and long-term outcomes in terms of fragility and mortality [3]. Mechanisms behind these detrimental relationships are not fully identified and are only partially mediated by chronic hyperglycemia exposure [3].

Neuropeptide-mediated circuits are implicated in several metabolic pathways [4]. Among them, they have been demonstrated to influence bone metabolism by controlling osteoblasts and osteoclasts' activity [5–8], engaging in a complex interplay with sex hormones, where oestrogen deficiency was shown to modify neuropeptide levels in the brain and bone, thus favouring postmenopausal osteoporosis [9].

Among the most investigated neuropeptides in metabolic diseases, neurotensin (NT), which has a role as both a neurotransmitter and gastrointestinal peptide, is a fine regulator of overall energy balance [10] and inflammatory processes [11, 12]. NT promotes lipid absorption through the gut in the presence of increased intestinal fat concentrations and enhances systemic and local pro-inflammatory processes [10]. Elevated concentrations of its precursor proneurotensin (proNT) are predictive of obesity, non-alcoholic fatty liver disease [13–15], cancer [16] and cardiovascular mortality [17], and these associations are more pronounced in women [18, 19].

As for type 2 diabetes, the existence of a strong correlation between proNT and impaired glucose-insulin metabolism has been reported in numerous studies [13–17]. Baseline proNT levels are associated with the presence of dysmetabolic features in obese children and predict further metabolic alterations, as impaired β-cell function to compensate for insulin-resistance, later in life [20]. In adults, elevated plasma proNT was shown to be associated with the presence of type 2 diabetes and poor glucose control [14, 15], whereas in non-diabetic individuals, predicted diabetes' onset over time [17], mostly in elderly female populations [13].

Indeed, several studies suggest a potential interplay between NT and the female gonadal axis. NT is expressed in follicle-stimulating hormone (FSH) and luteinising hormone (LH) positive cells of rats, and its expression levels vary across phases of the oestrous cycle [21]. NT levels also rose in the pituitary after ovariectomy-induced menopause [22]. Furthermore, NT is involved in cytokine secretion and inflammatory processes implicated in osteoporosis [23] and its receptor 3 Sortilin was shown to impact on calcification processes [24].

Despite all these data, the relationship between NT and bone metabolism remains an unexplored terrain. Our research aimed to assess the role of proNT in predicting impaired bone mineralisation and bone loss in postmenopausal women with type 2 diabetes.

2 | Materials and Methods

2.1 | Study Population

For this investigation, we analysed data from 126 women with type 2 diabetes recruited among those referred to the Diabetes outpatient clinics of Sapienza University, Rome, Italy, for diabetes management and care; forty-nine patients were re-evaluated after 12 months and data were available for follow-up analyses.

To be enrolled in this study, patients had to meet the following inclusion criteria: female subjects ≥ 18 years old; diagnosis of type 2 diabetes according to the American Diabetes Association 2009 criteria; body mass index (BMI) between 20 and 40 kg/m^2, body weight ≤ 120 kg; HbA1c < 7.5%; treatment with metformin in monotherapy at a stable dose for at least 12 weeks before enrolment; not on pregnancy. Main exclusion criteria were: recent/ongoing treatment with agents known to influence bone metabolism (e.g., bisphosphonates, calcitonin, corticosteroids or hormone replacement therapy), other/secondary causes of bone disease, substance abuse, clinically significant depression, or current psychiatric care. We also excluded from this study individuals with a current or history of therapy with antidiabetic agents other than metformin, to mitigate the potential risk of an impact of other antidiabetic therapies on bone metabolism/fracture risk.

All participants underwent clinical work-up, including weight and height measurements, BMI calculation, and systemic blood pressure assessment (mean value of three measurements recorded). Venous blood sampling was performed for metabolic evaluation, measuring fasting glycaemia (FBG, mg/dl), glycosylated haemoglobin (HbA1c, mmol/mol—%), total cholesterol (mg/dl), total cholesterol (mg/dL), high-density lipoprotein cholesterol (HDL, mg/dL), triglycerides (mg/dL), aspartate aminotransferase (AST, IU/L), alanine aminotransferase (ALT, IU/L), gamma-glutamyl transpeptidase (GGT, mg/dL), by standard laboratory methods. Low-density lipoprotein cholesterol (LDL, mg/dl) levels were obtained using the Friedewald formula. Insulin resistance was estimated using HOMA-IR. Circulating levels of bone and inflammatory markers such as parathormone (PTH), osteopontin (OPN), osteocalcin (OC), osteoprotegerin (OPG), sclerostin, IL-1β and TNF-α were measured by multiplex kits Milliplex (pg/mL; Merk Life Science S.r.l., Milan, Italy); serum N-terminal propeptide of type I procollagen (P1NP, μg/l) levels were assessed by ELISA kit.

The circulating concentration of proNT, a stable precursor fragment of NT released in equimolar amounts relative to NT, was quantified in plasma immediately frozen after separation and stored at −80°C. ProNT levels were assessed using a chemiluminometric sandwich immunoassay designed to detect proNT amino acids 1–117 (pmol/L), as previously described [25]. The analytical assay sensitivity was 4.8 pmol proNT/l, with an intra-inter-assay coefficient of variability < 5%.

2.2 | Bone Mineral Density (BMD) Assessment

We conducted an evaluation of the BMD in all study participants at the lumbar spine (L1–L4 anteroposterior) and hip (total

hip and femoral neck) levels by bone density scan (Dual-Energy X-ray Absorptiometry, DXA, Hologic Discovery [S/N 84191, Bedford, MA, USA]). All the examinations were carried out at the Bone Metabolism Service of Sapienza University of Rome, Italy, by an expert technician according to standardised procedures. The quantification of BMD was expressed in grams per square centimetre (g/cm^2). In accordance with World Health Organization (WHO) criteria [26], osteoporosis was diagnosed in subjects with a *T*-score ≤ -2.5, while osteopenia was assigned to those with a *T*-score ranging between -2.5 and -1.0. An evaluation of bone microarchitecture in our study participants was also performed, by integrating the DXA output with the trabecular bone score (TBS) measurement, by the TBS iNsight software, version 2.1.2.0, to site-matched spine scans. For the TBS assessment, validated cut-off values were used [27]: TBS > 1.31 denoting normal bone texture, TBS ranging between 1.23 and 1.31 indicative of partially degraded bone texture, and TBS < 1.23 suggesting degraded texture.

In forty-nine patients DXA scan was repeated after 12 months from the first evaluation and data were recorded for the analysis; none of these participants underwent any therapy or lifestyle change during the follow-up period.

2.3 | Statistics

Descriptive statistics are presented as mean ± standard deviation (SD) for continuous variables or percentage for categorical variables in the manuscript and tables. Differences between independent groups were compared using Student's *t*-test or Bonferroni-adjusted analysis of variance (ANOVA) for continuous variables and by χ^2 test for categorical variables. Correlations between proNT levels and clinical parameters were assessed by Spearman or Pearson's coefficient and linear univariate regression, as appropriate. To test the independence of the association between proNT and bone health parameters, we performed multivariable logistic and linear regression analyses at the baseline and after 1 year of follow-up, adjusted for traditional risk factors and parameters significantly associated with bone metabolism at the univariate test. p value < 0.05 was considered statistically significant. All statistical analyses were performed using IBM Corp. Released 2020. IBM SPSS Statistics for Macintosh, Version 27.0. Armonk, NY: IBM Corp.

2.3.1 | Sample Size and Power Calculation

This is the first study evaluating plasma proNT in relation to bone metabolism parameters. Therefore, a post hoc sample size calculation was performed in our study population entering circulating proNT levels found in individuals with normal BMD (n = 86) versus those with osteopenia/osteoporosis (n = 40) (proNT: 161.6 ± 108.8 vs. 200.8 ± 113.7 pg/mL, respectively; p = 0.01) and we obtained that our study had a statistical power = 96.1% with α-error = 0.05 to detect the association between proNT and low BMD [28].

2.4 | Ethics

This study protocol was approved by the local Ethics Committee of Sapienza University (Approval n. 493/19 Rif. 4951–July 7, 2019); the study was conducted in accordance with the Declaration of Helsinki, version 2013. All participants gave their informed consent before any study procedure.

3 | Results

Within our study population, 32% of women affected by type 2 diabetes had degraded bone mineralisation: 37 patients had osteopenia and three osteoporosis, whereas 86 women with type 2 diabetes had normal BMD at the DXA evaluation. Patients with osteopenia/osteoporosis had significantly higher proNT levels than those with normal *T*-score (proNT: 200.8 ± 113.7 vs. 161.6 ± 108.8 pg/mL, respectively; $p = 0.013$). Characteristics of study participants according to their bone health status are reported in Table 1.

Plasma proNT inversely associated with BMD and *T*-score measured at the total ($r = -0.25$, $p = 0.005$; $r = -0.25$, $p = 0.006$), and neck femur ($r = -0.20$, $p = 0.024$; $r = -0.20$, $p = 0.025$) level. Furthermore, a direct association was found between proNT and OPN ($r = 0.18$, $p = 0.042$), P1NP ($r = 0.32$, $p < 0.001$), TNF-α ($r = 0.225$, $p = 0.012$) and IL-1β ($r = 0.32$, $p = 0.002$).

Finally, we also investigated the relationship between proNT and bone microarchitecture quality, assessed by TBS at the lumbar column level, finding that greater proNT was associated with worse TBS in our study participants ($r = -0.20$, $p = 0.027$).

ProNT progressively increased in the presence of a lower estimated glomerular filtration rate and longer diabetes duration; no significant association was shown with age or other clinical parameters, as reported in Table 2.

Having osteopenia/osteoporosis was significantly associated with higher circulating levels of proNT (β: −4.665, $p = 0.007$) at the univariate regression analysis, and with age (β: 0.047, $p = 0.022$), lower BMI (β: −0.18, $p = 0.001$), and circulating OPN (β: 0.00, $p = 0.036$) and OPG (β: 0.002, $p = 0.030$). Conversely, no association was found between the diagnosis of osteopenia/osteoporosis and other clinical parameters, such as kidney function, glucose control or other biomarkers of bone metabolism or inflammation. ProNT levels at the baseline were independently associated with the presence of osteopenia/osteoporosis with an OR of 8.29 (95% CI, 1.5–45.8; $p = 0.015$) at the multivariable logistic regression analysis adjusted for age, BMI, HbA1c, smoking status, physical activity, 25(OH) vitamin D levels, and eGFR (Table 3.)

In the 49 patients with type 2 diabetes who were re-evaluated with DXA scan and metabolic profiling after 12 months, the follow-up total femur *T*-score inversely correlated with baseline proNT ($r = -0.36$, $p = 0.011$) and OPN ($r = -0.302$, $p = 0.037$) levels, and with age ($r = -0.374$, $p = 0.008$) and diabetes' duration ($r = -0.311$, $p = 0.030$). No relationship was found between the follow-up total femur *T*-score and other clinical or

TABLE 1 | Characteristics of patients with type 2 diabetes according to the presence/absence of osteoporosis/osteopenia.

	Normal BMD $n = 86$	Osteopenia/osteoporosis $n = 40$	p-value
Age (years)	64.9 ± 10.67	69.56 ± 9.26	0.015
Diabetes' duration (years)	7.35 ± 6.24	8.47 ± 6.25	0.18
BMI (kg/m^2)	29.44 ± 4.04	26.805 ± 3.80	0.001
Waist circumference (cm)	101.378 ± 10.84	97 ± 11.44	0.039
SBP (mmHg)	131.50 ± 13.33	124.75 ± 8.69	0.001
DBP (mmHg)	80.50 ± 8.14	78.25 ± 6.56	0.13
FBG (mg/dl)	117.88 ± 20.22	114.37 ± 21.81	0.41
HbA1c (%—mmol/mol)	6.43 ± 0.53	6.45 ± 0.54	0.91
Total cholesterol (mg/dl)	180.05 ± 35.19	171.81 ± 36.02	0.21
HDL-cholesterol (mg/dl)	56.12 ± 12.04	51.57 ± 10.97	0.06
LDL-cholesterol (mg/dl)	99.02 ± 30.98	96.97 ± 35.28	0.81
Triglycerides (mg/dl)	135.53 ± 61.87	126.97 ± 56.09	0.49
AST (IU/l)	21.93 ± 9.36	22.60 ± 10.47	0.76
ALT (IU/l)	22.82 ± 11.12	21.53 ± 9.92	0.59
GGT (IU/l)	23.92 ± 13.35	36.00 ± 31.74	0.35
eGFR (ml/min/1.73 m^2)	96.09 ± 20.72	96.46 ± 21.14	0.93
TSH	2.54 ± 2.22	1.85 ± 1.19	0.53
Total femur BMD (g/cm^2)	0.939 ± 0.092	0.747 ± 0.056	< 0.001
Total femur T score	−0.02 ± 0.75	−1.60 ± 0.46	< 0.001
Femur neck BMD (g/cm^2)	0.769 ± 0.10	0.621 ± 0.05	< 0.001
Femur neck T score	−0.685 ± 0.90	−2.01 ± 0.49	< 0.001
Lumbar spine BMD (g/cm^2)	1.0 ± 0.14	0.90 ± 0.12	< 0.001
Lumbar spine T score	−0.34 ± 1.27	−1.34 ± 1.15	< 0.001
25(OH) vitamin D (ng/mL)	27.38 ± 14.55	32.97 ± 21.81	0.26
PTH	48.68 ± 40.80	60.10 ± 42.99	0.09
OPN (pg/mL)	14,378 ± 9477.35	18,600.60 ± 10,648.55	0.03
OPG (pg/mL)	515.45 ± 192.71	617.75 ± 309.76	0.11
OC (pg/mL)	8037.38 ± 5132.68	9115.61 ± 5270.67	0.21
Sclerostin (pg/mL)	1385.89 ± 1473.37	1473.37 ± 2343.84	0.68
P1NP (μg/L)	30.32 ± 11.73	34.47 ± 21.26	0.60
Serum calcium	9.42 ± 1.42	8.99 ± 1.65	0.21
ProNT (pg/mL)	161.58 ± 108.84	200.81 ± 113.71	0.013

Note: Student's t-test for mean comparison applied.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMD, bone mineral density; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FBG, fasting blood glycaemia; GGT, gamma glutamyl transferase; HDL, high density lipoprotein; LDL, low density lipoprotein; OC, osteocalcin; OPG, osteoprotegerin; OPN, osteopontin; P1NP, Procollagen type 1 N-terminal propeptide; ProNT, proneurotensin; PTH, parathyroid hormone; SBP, systolic blood pressure; TSH, thyroid stimulating hormone.
p-value < 0.05 considered statistically significant.

metabolic parameters. Comparisons between clinical characteristics at baseline versus follow-up visits are reported in Table 4.

Finally, at the multivariable linear regression analysis, baseline proNT levels were demonstrated to independently predict the 12-month total femur T-score after adjusting for basal femur T score, age, BMI, eGFR, HbA1c, 25(OH)D and OPN levels (proNT $p = 0.023$; R^2 of the model: 0.96; Table 5).

4 | Discussion

The main result of this prospective study is the identification of a relationship between proNT levels and reduced bone mineralisation in women with type 2 diabetes; in this population, proNT concentration represented an independent predictor of further BMD decline after 1 year. Circulating proNT was also associated with degraded bone architecture (TBS) and with serum biomarkers of bone remodelling and pro-inflammatory state within

TABLE 2 | Correlates of plasma proNT levels.

Parameter	Correlation's coefficient	*p*-value
Age	0.150	0.090
Diabetes' duration	0.243	0.006
BMI	−0.075	0.404
Waist circumference	−0.036	0.688
SBP	−0.117	0.191
DBP	−0.142	0.113
FBG	−0.013	0.884
HbA1c	−0.086	0.339
Total cholesterol	−0.020	0.833
HDL-cholesterol	−0.031	0.756
LDL-cholesterol	0.093	0.383
Triglycerides	−0.092	0.335
AST	−0.02	0.997
ALT	−0.11	0.327
GGT	0.025	0.849
eGFR	−0.196	0.041
Total femur BMD	−0.248	0.005
Total femur *T* score	−0.246	0.006
Femur neck BMD	−0.202	0.024
Femur neck *T* score	−0.202	0.025
Lumbar spine BMD	0.05	0.603
Lumbar spine *T* score	0.049	0.582
25(OH) vitamin D	−0.126	0.163
PTH	−0.099	0.285
Serum calcium	0.214	0.016
OPN	0.18	0.042
OPG	0.078	0.388
OC	−0.009	0.919
Sclerostin	0.021	0.826
P1NP	0.317	0.001
TNF-α	0.225	0.012
IL1b	0.32	0.002

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FBG, fasting blood glycaemia; GGT, gamma glutamyl transferase; HDL, high density lipoprotein; IL1b, Interleukin 1β; LDL, low density lipoprotein; OC, osteocalcin; OPG, osteoprotegerin; OPN, osteopontin; P1NP, Procollagen type 1 N-terminal propeptide; PTH, parathyroid hormone; SBP, systolic blood pressure; TNF-α, Tumour Necrosis Factor α.

our study population. However, systemic inflammation was not an indicator of reduced bone mineralisation per se and did not contribute to explain BMD modification during the follow-up in our female cohort with type 2 diabetes. The association between proNT and impaired bone mineralisation observed in the present study was neither influenced by patients' phenotype, or kidney function nor by the presence of additional dysmetabolic disorders.

The existence of a tight relationship between type 2 diabetes and bone fragility has been widely described, but the underlying mechanisms behind this association remain only partially understood. Besides classical pathways and traditional risk factors, data from the literature show that neuropeptide-mediated circuits influence bone metabolism, constituting the so-called 'brain-bone axis' [29]. Neuropeptides can regulate the sympathetic activity, oestrogens' effects and endocrine axes implicated in obesity and metabolic diseases; all these pathways ultimately converge on the regulation of bone metabolism [4–8, 30, 31]. Among them, a central role in skeletal homoeostasis is played by those neuropeptides which serve both as regulators of the energy balance in the central nervous system and as gastrointestinal peptides, such as secretin and neuropeptide Y (NPY) [8]. Recently, central secretin levels were demonstrated to influence bone mass accrual by modulating the sympathetic tone, and secretin expression levels in the ventromedial hypothalamus are a determinant of osteopenia development [30]. Similarly, the expression levels of the NPY receptors NPYR2 and NPYR6 in the central nervous system have been associated with osteoblast and osteoclast activity, osteoblast precursor survival and overall bone mineralisation rate in experimental models [31].

Dual functionality as a central neurotransmitter and gastrointestinal peptide is fully attributed to NT, a small peptide that mediates leptin circuits regulating appetite, and physical activity, and is involved in primary homoeostatic functions, that is, blood pressure support, thermogenesis, pain and sleep control [10]. However, the major metabolic regulation exerted by this neuropeptide is correlated with its activity as a gastrointestinal peptide. NT is secreted by the intestinal neuroendocrine cells in response to high-fat ingestion and promotes lipid absorption through the gut; its circulating concentration rises after meals, parallelled by serum triglycerides and bile acid levels [32]. Once released, NT regulates the secretion of other gastrointestinal peptides via endocrine and paracrine circuits; a co-secretion of NT and glucagon-like peptide 1 (GLP-1) was shown in some investigations [33]. Conversely, fasting NT concentrations were found to be at least partially mediated by NT gene polymorphisms in our recent work [34]. Increased circulating levels of its precursor proNT are highly predictive of obesity development, type 2 diabetes, cancer, and overall cardiovascular mortality [13–19]. ProNT is associated with adverse outcomes in cardiovascular disease, particularly in women [17, 18], where it is also a marker for decreased survival rates in breast cancer [19].

In the present study, proNT exhibited a role as an independent risk factor for impaired bone mineralisation and further decline during the follow-up. The association between circulating NT and bone metabolism has not been investigated previously and further studies are warranted to unravel the mechanistic pathways behind this relationship. However, several potential mechanisms might be likely implicated in this association. First, NT is centrally involved in inflammatory processes and cytokine secretion; it is released by mast cells and its secretion can, in turn, activate them, along with lymphocytes and macrophages [35]; a specific role of mast cells in the pathogenesis of primary and secondary osteoporosis has been demonstrated [23]. Furthermore, the NT/NT receptor axis modulates adipogenesis and is involved in adipose tissue inflammation. NT-knocked-out mice exposed to hypercaloric and fat-rich diets preserved the structural integrity of the adipose tissue, which translated into better glucose tolerance and insulin sensitivity [36]. Thus, a

TABLE 3 | Multivariable logistic regression analysis investigating the determinants of osteopenia/osteoporosis at the baseline.

					95% confidence interval	
	β	Standard error	*p*-value	Odds ratio	Lower	Upper
Pro-NT	2.115	0.872	0.015	8.292	1.502	45.780
Age	0.035	0.025	0.172	1.035	0.985	1.088
25(OH) vitamin D	0.022	0.013	0.095	1.022	0.996	1.048
BMI	−0.142	0.067	0.035	0.868	0.761	0.990
Smoking status	0.327	0.261	0.211	1.387	0.831	2.314
Physical activity	0.395	0.327	0.228	1.484	0.782	2.817
eGFR	0.002	0.006	0.765	1.002	0.990	1.014

Note: The presence of osteopenia/osteoporosis versus normal BMD is the dependent variable.
Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; ProNT, Proneurotensin.

TABLE 4 | Clinical characteristics of type 2 diabetes patients undergoing DXA follow-up; $n = 49$.

	Baseline	Follow-up	*p*-value
BMI (kg/m^2)	28.45 ± 3.5	28.11 ± 3.6	0.14
Waist circumference (cm)	99.11 ± 10.9	98.62 ± 10.9	0.70
FBG (mg/dl)	116.37 ± 20.5	113.5 ± 20.2	0.29
HbA1c (%—mmol/mol)	6.4 ± 0.4	6.4 ± 0.8	0.94
Total cholesterol (mg/dl)	173.61 ± 31.5	172.03 ± 37.1	0.71
HDL-cholesterol (mg/dl)	54.76 ± 12.8	54.85 ± 14.3	0.95
LDL-cholesterol (mg/dl)	95.48 ± 30.9	92.24 ± 35.7	0.50
Triglycerides (mg/dl)	130.69 ± 49.8	124.50 ± 52.1	0.34
25(OH) vitamin D (ng/mL)	28.07 ± 15.4	29.45 ± 14.7	0.56
Total femur BMD (g/cm^2)	0.88 ± 0.10	0.87 ± 0.11	0.016
Total femur *T* score	−0.537 ± 0.85	−0.663 ± 0.88	< 0.001
Femur neck BMD (g/cm^2)	0.726 ± 0.10	0.720 ± 0.10	0.23
Femur neck *T* score	−1.081 ± 0.90	−1.177 ± 0.92	0.025
Lumbar spine BMD (g/cm^2)	0.972 ± 0.14	0.971 ± 0.14	0.72
Lumbar spine *T* score	−0.663 ± 1.3	−0.667 ± 1.3	0.90

Abbreviations: BMD, bone mineral density; BMI, body mass index; FBG, fasting blood glycaemia; HDL, high density lipoprotein; LDL, low density lipoprotein.

TABLE 5 | Multivariate linear regression analysis investigating determinants of total femur *T*-score at the 12-month follow-up.

	Non standardised β coefficient	Standard error	Standardised β coefficient	*p*-value
Baseline total femur score	0.947	0.045	0.933	< 0.001
Age	−0.006	0.005	−0.071	0.198
ProNT	−0.001	0.000	−0.094	0.023
BMI	−0.010	0.010	−0.045	0.294
HbA1c	−0.129	0.087	−0.060	0.148
eGFR	0.001	0.002	0.019	0.709
25(OH) vitamin D	0.003	0.002	0.057	0.174
OPN	−0.095	0.099	−0.038	0.344

Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; OPN, osteopontin; ProNT, Proneurotensin.

higher proNT concentration might also modulate the bone marrow microenvironment, by favouring a pro-inflammatory milieu and promoting adipogenesis, thus negatively impacting osteogenesis. Moreover, the NT receptor 3, named Sortilin, has been shown to impact calcification processes; its tissue expression increases with ageing and is associated with atherosclerotic calcification [24], cellular senescence and osteoarthritis [37]. Finally, NT contributes to the action of corticotropin-releasing

hormone (CRH) to mediate several oestrogen-dependent pathways [38], and proNT is described as a quasi-gender-specific marker of cardiovascular mortality [18, 19]. Thus, it is plausible that this neuropeptide could also influence other gender-associated diseases, such as bone fragility and osteoporosis.

Chronic hyperglycemia exposure has been implicated in the relationship between diabetes and bone fragility [3, 39]. Individuals recruited for this investigation were all in good glycaemic control with metformin alone as antidiabetic therapy. As for inclusion criteria, none of them were treated with anti-osteoporotic agents or had comorbidities or ongoing treatment known to affect bone health directly or indirectly. Thus, we might likely exclude the influence of these potential confounders in determining bone status in the study population. Furthermore, all participants underwent comprehensive metabolic phenotyping, including measurement of several bone markers, in addition to DXA scans, thereby offering an integrated assessment of bone status, coherently converging on a strong association between impaired bone status and proNT in these patients.

In conclusion, this study highlights, for the first time, the importance of considering neuroendocrine factors, and specifically the NT signalling, in the relationship between bone metabolism and type 2 diabetes. Further investigation into the role of NT in bone health may yield insights into the pathophysiology of osteoporosis in this population and put the basis for targeted interventions to mitigate fracture risk and improve outcomes in the presence of diabetes.

Author Contributions

I.B., M.G.C. and M.G.B. conceptualised and designed the study. M.G.C., S.M., E.R. and A.L. contributed to data interpretation, reviewed the results and revised the manuscript. S.D., G.P., F.A.C., V.V., E.B., and T. F. were responsible for patients' recruitment and data collection. I.B., M. G.C. and M.G.B. performed the data analysis. F.S. and O.M. conducted the laboratory procedures. All authors have read and approved the final manuscript. The corresponding author confirms that all listed authors meet the authorship criteria.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Peer Review

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