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Bone quality is associated with body composition measurements in patients with type 2 diabetes.

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Introduction

Osteoporosis is a chronic disease of bone tissue, characterized by a loss of bone mineral density (BMD) leading to a decline of the bone strength and ultimately to increase the risk of fractures [1].

It represents a major public health problem due to high morbidity and mortality issues [2]. Over 200 million people worldwide suffer from osteoporosis [3] and it is estimated that one in three women and one in five men are at risk of an osteoporotic fracture [4,5].

Type 2 diabetes mellitus (T2DM) is a pandemic metabolic disease with affected people estimation rising to 438 million by the year 2030; the global prevalence of diabetes has been steadily increasing over the past few decades, reaching a number of about 422 million, and 1.6 million deaths are directly ascribed to diabetes every year [6,7].

Both T2DM and osteoporosis are affected by aging and lifestyle and quite often coexist [8]. Several lines of evidences suggest an increased fracture risk in T2DM that is strictly associated with longer disease duration, poor glycemic control, and diabetic related complications [9].

In a prospective study of 32,089 postmenopausal women, the Iowa Women's Health Study, the risk of hip fractures was 1.7 times higher among T2DM patients, after adjusting for several risk factors [10] and accordingly, in the Women's Health Initiative Observational Study, including 93,000 postmenopausal women, of whom 5,285 subjects had T2DM, a significantly higher risk of fracture in several sites was detected in T2DM women, after correcting for multiple risk factors, including previous history of falls [11]. Similar data were observed in the longer follow-up (22 years) of the Nurses' Health Study, showing an increased risk both in type 1 diabetes mellitus (T1DM) (n= 292) and T2DM (n= 8348) [RR: 2.2 (95% CI, 1.87–2.7) [12]. Furthermore, the risk of vertebral fractures in patients with diabetes has been shown to be up to 2.03-fold higher than in non-diabetic controls [13].

The pathophysiology of T2DM related bone fragility includes the hyperglycemia, the oxidative stress, the accumulation of advanced glycation end-products (AGEs) that impairs collagen

properties, the increased marrow adiposity, the role of adipokines, but also the treatment-induced hypoglycaemia, the contribute of certain antidiabetic medications with a direct effect on bone and mineral metabolism (such as thiazolidinediones), and also an increased propensity for falls [9,14].

Measurement of the BMD with dual-energy X-ray absorptiometry (DXA) scans is the gold standard tool to diagnose osteoporosis [2]. However, T2DM patients show similar or even higher BMD values than that of the non-diabetic counterpart at DXA examination, highlighting the discrepancies between BMD and fracture risk and suggesting that measuring BMD may not reflect bone fragility of these patients [14, 15]. The Fracture Risk Assessment tool (FRAX), a widely used fracture risk assessment tool, which estimates the probability for major osteoporotic fracture (hip, clinical spine, humerus, or forearm) and hip fracture over 10 years, has been shown to improve fracture prediction over *T*-score alone [16]. Unfortunately, its output underestimates fracture risk in T2DM patients, especially when disease is of long duration [17, 18], in contrast to good concordance with the fracture rate observed in patients without T2DM [19].

Thus, the common work-up to detect fracture risk seems to be not completely able to identify higher fracture risk in T2DM.

T2DM is usually associated with body composition modifications, including increase of fat and reduction of muscle mass [20]. These changes could contribute to fracture risk, but the association between body composition has been poorly investigated in T2DM [21,22].

Recently, the Trabecular Bone Score (TBS) has been introduced as a new texture parameter coming from pixel gray-level variations in DXA images at lumbar spine. It has been proposed to reflect bone microarchitecture status and cumulating evidences suggest that it may contribute to fracture risk assessment [23-26]. Reduced lumbar spine TBS values have been reported in a number of studies on T2DM subjects [27-30].

Beyond DXA evaluation, it was also previously shown that quantitative ultrasound (QUS) of bone could be a valuable tool to explore surrogate markers of bone quality assessing fracture risk in several settings of patients including T2DM subjects [31-35].

Since the relationship between body composition with TBS and QUS measurements has not fully investigated in T2DM, the aim of our research was to explore fracture risk by considering an integrative assessment of bone health to possibly identify predictors of bone fragility.

Materials and Methods

Participants

This cross-sectional investigation included a group of Caucasian subjects with T2DM, consecutively referred to the Diabetic Outpatients Clinic of the Department of Clinical and Experimental Medicine, University Hospital of Messina (Messina, Italy). Patients were included if they had an age > 50 years, time since diagnosis of T2DM > 5 years, no changes in diabetic medical treatment over the latest 12 months and a full screening for diabetic related complications over the latest 6 months.

Conversely, exclusion criteria were history of cancer, moderate to severe kidney or liver failure, heart failure with New York Heart Association (NYHA) functional classification >2, moderate and severe respiratory failure, endocrine disorders of thyroid, parathyroid or adrenal glands, dementia, known psychiatric condition or use of psychotropic drugs, use of active bone agents such as bisphosphonates, denosumab, selective estrogen receptor modulators, strontium ranelate or teriparatide.

The study was approved by the local Ethics Committee with protocol n. 71/2019; it was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments and written informed consent was obtained from all the participants before entering the study.

Clinical evaluation

Height and weight measurements were taken from eligible participants at baseline according to standard procedures and BMI was calculated as weight in kilograms divided by the square of height in meters (Kg/m^2). Data related to the presence or not of diabetic complications were recorded: particularly, macrovascular disease was defined as a history of a cardiovascular event and/or ischemic electrocardiogram abnormalities at rest or in a stress test, or the presence of plaques at the ultrasonography of the carotid arteries or the peripheral arterial vessels or as the presence of an intima media thickness greater than 1.5 mm; sensory-motor neuropathy was diagnosed by

vibration perception test, by monofilament pressure sensation test or by electromyography; retinopathy was detected by high-quality fundus photographs.

Metabolic control was assessed taking into account the mean value of glycated hemoglobin (HbA1c) of the latest 12 months.

Fracture risk assessment was estimated by Fracture Risk Assessment Tool (FRAX®), which is a computer-based algorithm (http://www.shef.ac.uk/FRAX) that calculates the 10-year probability of a major fracture (hip, clinical spine, humerus or wrist fracture) and the 10-year probability of hip fracture. In accordance to FRAX, calibrated for Italian subjects, fracture risk was derived from by age, BMI and dichotomized risk factors comprising prior fragility fracture, parental history of hip fracture, current tobacco smoking, exposure to oral glucocorticoids, rheumatoid arthritis, other causes of secondary osteoporosis and alcohol consumption. FRAX score was calculated without considering BMD.

Muscle strength was assessed by measurement of handgrip using a Jamar dynamometer, according to a standardized protocol consisting in three consecutive grip strength measurements with the second handle position of the device for each hand, with a rest period of 30" between successive attempts. Maximal handgrip strength was recorded.

Bone status assessment

A Dual-energy X-ray Absorptiometry (DXA) densitometer (Hologic Discovery) was used to assess BMD at the lumbar spine (L1-L4) and at the femoral neck. Our DXA densitometer was calibrated on a daily basis according to the manufacturer's instruction and its coefficient of variation (CV) was 0.5% with the standard phantom.

The Trabecular Bone Score (TBS) was also calculated through DXA images by iNsight software (version 3.0; Medimaps group, Geneva, Switzerland). TBS was evaluated considering the variogram of the trabecular bone projected image, calculated as the sum of the squared gray-level differences between pixels at a specific distance and angle. TBS was then calculated as the slope of

the log-log transform of this variogram. TBS was reported as the mean value of the individual measurements for lumbar spine TBS.

To further assess bone status we also performed Quantitative UltraSound (QUS) measurements at the proximal phalangeal metaphysis of the last four fingers of the non-dominant hand using a DBM Sonic Bone Profiler (Igea, Carpi, Italy) as previously described [32]. Amplitude Dependent Speed of Sound (AD-SoS), Bone Transmission Time (BTT), Fast Wave Amplitude (FWA), Signal Dynamic (SDy) and the derived Ultrasound Bone Profile Index (UBPI) (UBPI= -(0.0018 x SDy - 0.0560 x FWA 0.0560 - 1.1467 x BTT + 3.0300)) were the studied QUS variables.

Vertebral fractures

Vertebral fractures (Vfs) were ascertained by vertebral morphometry at a lateral scan of thoracic and lumbar spine. Vfs was defined in accordance to the semiquantitative method proposed by Genant: a fracture was diagnosed if a vertebra body had at least a 20% height reduction in the anterior, middle or posterior height compared with the same or adjacent vertebra [36].

Body composition

A whole body Dual-energy X-ray Absorptiometry (Hologic Discovery) scan was used to assess body composition (percentage fat mass and lean mass) [37]. Adipose tissue indices (total body % fat, fat mass/height^2, android/gynoid ratio, % fat trunk/% fat legs, trunk/legs fat mass ratio) and lean indices (lean/height^2 and appendicular lean/height^2) were considered.

Statistical analysis

Data were reported as means ± SD or median (IQR) for continuous variables and percentages for categorical variables. The normal distribution of values was confirmed by the Kolmogorov-Smirnov test. Student's t-test for unpaired observations and Mann-Whitney test were used as appropriate. Fisher's exact test was used to calculate differences in categorical variables. The degree of association between two variables was verified by Spearman's coefficient. Multiple regression analysis was performed to analyze the relationship between a dependent variable and one

or more independent variables. All reported p values were two-sided, and values of $p<0.05$ were considered to indicate statistical significance.

Results

A total of forty-five patients [median age 67 (60 to 70)] were recruited and their main clinical characteristics are shown in **Table 1**.

Participants were mostly females, overweight or obese subjects, with a mean value of HbA1c in the latest 12 months equal to 6.94% ±0.77% SD. Women showed a higher BMI than men [32.4 (25.8 to 36.4) vs. 27.5 (23.15 to 30.4), p=0.03]. Up to 20% of recruited subjects reported prevalent fractures; in particular, morphometric vertebral fractures were detected in 17% of patients without any gender differences (males vs. females, p=0.6). The median 10-year probability of fractures was 8.1% and 2.3% for major osteoporotic fracture and hip fracture respectively (**Table 1**).

Phalangeal bone-ultrasound measurements are shown in **Table 2**.

BTT measurements were significantly different in T2DM subjects with and without prevalent fractures [0.93 (0.7 to 1.15) vs. 1.27 (0.99 to 1.45) respectively, p=0.03].

BMD at lumbar spine and femoral site and related T-score and Z-score values are reported also in Table 2. According to the WHO recommendation for the diagnosis of osteoporosis, by considering the median value of femoral neck T-score, participants fell in the range of osteopenia. Conversely, the median T-score value at lumbar spine was in the normal range. The median TBS value was 1.28, thus consistent with a partially degraded microarchitecture (TBS deterioration grade 1: TBS >1.350, grade 2: 1.350-1200, grade 3: <1.200) [38,39].

The DXA measurements of adipose and lean indices are reported in **Table 3**. As shown, adipose mass was representative of ~ 40% of body composition. The median handgrip strength of the participants was 22.3 (18.9 to 31.3) and was indicative of a slight reduced functional status [40].

Body composition measurements were significantly related with phalangeal ultrasound outcomes: BTT was negatively associated with total body fat; moreover, BTT and UBPI were significantly associated with fat distribution according to android/gynoid ratio (**Table 4**). BMD at

lumbar spine was positively associated with lean indices (lean/height^2 and appendicular lean/height^2). Differently, TBS values were negatively related with adipose indices (total body % fat and fat mass/Height^2) (**Table 5**). A borderline negative significant association was observed between TBS values and metabolic control in the 8 participants with VFs (r= -0.36, p= 0.07).

Handgrip strength was negatively related with both total body %fat and fat mass/height^2 (r= -0.75, p<0.001 and r= -0.7, p<0.001, respectively), it was also positively associated with android/gynoid ratio (r= 0.67, p<0.001), %fat trunk/%fat legs (r= 0.49, p=0.01) and appendicular lean/height^2 (r=0.48, p=0.001).

Finally, at multiple regression analysis, mean HbA1c value was independently associated with the QUS variable BTT (β= -0.34, SE 0.14, p=0.02), after correcting for age, time since T2DM diagnosis, adipose and lean indices. HbA1c was also independently associated with lumbar spine BMD (β= -0.1, SE 0.04, p=0.04), after correcting for the same variables. Handgrip strength was predictive of both lumbar (β= 0.009, SE 0.0034, p=0.01) and femoral neck BMD values (β= 0.006, SE 0.002, p=0.01).

Age (β= -0.008, SE 0.002, p=0.007) and hand grip strength (β= 0.01, SE 0.002, p=0.0001) were independently associated with TBS score, after correcting for lean and adipose indices, mean HbA1c values, time since T2DM diagnosis.

Discussion

Type 2 diabetes mellitus is burdened by high rate of fractures, but the currently standard diagnostic tools have not been able to fully identify diabetic patients at higher fracture risk to date [41]. Our findings provide new insights about body composition and surrogate markers of bone quality detected by QUS and TBS in these patients.

We found that one in five participants showed at least one prevalent fracture, and previously undiagnosed morphometric Vfs were found in 18% of subjects. Our findings are consistent with literature data suggesting high fracture risk in T2DM, although the risk of vertebral fracture has been recently reported to be not significantly higher than the non-diabetic counterparts [42]. A large amount of participants were overweight, and particularly females showed a median BMI in the range of obesity. As known, most of the available evidence supports a lower risk of proximal femur and vertebral fracture in obese adults, however, fracture risk in obesity is not lower at all skeletal sites [43,44]. As known, adipose tissue is associated with low grade inflammation that *per se* could increase fracture risk overtime. Particularly, pro-inflammatory cytokines from visceral fat such as interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) increase bone resorption, so may have harmful effects on BMD [45].

In general population and in T2DM patients fractures could occur even in patients with T-score values within the normal range [46]. Thus, to further investigate bone fragility beyond BMD, we evaluated a novel score named TBS, which evaluates pixel gray level variations in the projected lumbar spine DXA image and represents an indirect measure of bone quality that is an independent predictor of fracture [23].

As expected, TBS was positively associated with BMD at lumbar spine and femoral neck, but it was also negatively related to adipose indices, suggesting bone quality may depend from body composition, irrespective of BMI value. These data are consistent with the 2019 International Society for Clinical Densitometry (ISCD) Official Position that considered TBS a score associated

with major osteoporotic fracture risk in postmenopausal women with T2DM [47]. In addition, in the subset of participants with prevalent Vfs, we were able to identify a significant association between TBS and metabolic control, the worst metabolic control being associated with the worst TBS values. This result is in agreement with the relevance of glycemic control over time on bone health [48,49].

Our study confirmed several correlations between QUS measurements at phalangeal site and BMD, as well as with body composition features [49,50]; the 10-year probability of clinical vertebral fractures was also associated to QUS. QUS at phalangeal site was proven to be a reliable tool to screen for osteoporosis and to predict incident fractures independently from DXA; indeed, ultrasound transmission through bone depends on physical properties of bone tissue not detected by DXA, but that are able to modify bone strength and consequently the risk of fractures. BTT values were inversely related to body fat, but also associated to fat distribution and the prevalence of android distribution was associated with a higher BTT, once again our results underlining a better bone quality when lower fat mass with android distribution exists. Differently from BMD, BTT was able to discriminate T2DM with prevalent fractures. BTT was also predicted by metabolic control, and this is consistent with our previous findings in subjects with T1DM [49].

In our study, lean mass indices were positively associated with BMD at lumbar spine, but also with muscle performance as highlighted by handgrip strength. Muscle mass and strength were previously associated with bone health and fracture risk [51]. Muscle mass loss is often observed in T2DM [52], due to a reduced insulin-mediated glucose uptake in skeletal muscle and chronic inflammation, resulting in impaired energy production, weaker contraction of the muscle and muscle protein breakdown [53]. After considering the TBS as a dependent variable, for the first time, at multiple regression analysis we identify the predictive value of handgrip strength, thus we hypothesized that muscle performance could be associated with bone strength.

We acknowledge this study has some limitations as the cross-sectional design and the small sample size. Although the antidiabetic drugs were not included in the statistical analysis, we

selected patient assuming only metformin in most of cases. At the same time, to the best of our knowledge, this is the first report of an association between DXA body composition parameters and surrogate of bone strength in T2DM. Further controlled study and longitudinal analyses are warranted to better recognize fracture risk in T2DM.

Conclusions

T2DM is associated with increased fracture risk. Beyond BMD, DXA derived body composition assessment, TBS at lumbar spine and phalangeal QUS measurements provide information about bone strength as well as handgrip strength.

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APPENDIX

Supplementary Material:

- **Table 1:** Main clinical characteristics of T2DM participants.
- **Table 2:** Bone health evaluation by dual X-Ray absorptiometry (DXA) and phalangeal Quantitative UltraSound (QUS).
- **Table 3:** DXA evaluation of body composition in patients with T2DM.
- **Table 4:** Correlation coefficients (r) between DXA derived adipose and lean indices with phalangeal quantitative ultrasound (QUS) measurements of bone.
- **Table 5:** Correlation coefficients (r) between DXA derived measurements.

Table 1. Main clinical characteristics of T2DM participants.

	T2DM *(n=45)*
Age *(yr.)*	67 (60 to 70)
BMI *(kg/m^2)*	29.3 (25.6 - 33)
Sex *(%females)*	65
Waist to Hip Ratio	0.95 (0.92 to 0.99)
Time since diagnosis (*yr.)*	11 (6 to 15)
Diabetic related complication *[n(%)]*	
- Macroangiopathy *[n(%)]*	31 (69)
- Microangiopathy *[n(%)]*	8 (17)
- Neuropathy *[n(%)]*	3 (7)
Patients with prevalent fractures *[n(%)]*	9 (20)
Patients with prevalent VFs *[n(%)]*	8 (18)
Ten year probability of fractures	
- Major osteoporotic fractures *(%)*	8.1 ± 5
- Hip fracture *(%)*	2.31 ± 2

Values are expressed as median (IQR) or mean (SD) as appropriate. BMI= Body Mass Index; WHR= Waist to Hip Ratio; Vfs=Vertebral fractures.

Table 2. Bone health evaluation by dual X-Ray absorptiometry (DXA) and phalangeal Quantitative UltraSound (QUS).

DXA measurements	
L1-L4 BMD *(g/cm^2)*	0.91 (0.9 to 1.03)
L1-L4 T-score *(SD)*	-0.8 (-1.5 to -0.1)
Femoral neck BMD *(g/cm^2)*	0.73 (0.64 to 0.79)
Femoral neck T-score *(SD)*	-1.1 (-1.8 to -0.5)
TBS	1.28 (1.2 to 1.31)
QUS measurements	
AD-SoS *(m/s)*	1649 (1605 to 1788)
UBPI *(U)*	0.14 (0.09 to 0.22)
BTT *(μs)*	1.23 (0.92 to 1.3)

Values are expressed as median (IQR). BMD= Bone Mineral Density; TBS= Trabecular Bone Score; AD-SoS= Amplitude Dependent Speed of Sound (AD-SoS); UBPI= Ultrasound Bone Profiler Index; BTT= Bone Transmission Time.

Table 3. DXA evaluation of body composition in patients with T2DM.

	T2DM *(n=45)*
Adipose Indices	
Total body % Fat	39.5 (32.7 to 42.4)
Fat mass / Height^2 *(Kg/m^2)*	11.2 (8.8 to 13.4)
Android / Gynoid ratio	1.13 (1.02 to 1.28)
%Fat trunk / %Fat legs	1.1 (0.99 to 1.2)
Trunk/Legs fat mass ratio	1.35 (1.17 to 1.5)
Lean Indices	
Lean/Height^2 *(Kg/m^2)*	17.7 (16.59 to 18.5)
Appen. Lean/Height^2 *(Kg/m^2)*	7.05 (6.64 to 7.45)

Values are expressed as median (IQR).

Table 4. Correlation coefficients (r) between DXA derived adipose and lean indices with phalangeal quantitative ultrasound (QUS) measurements of bone.

	Phalangeal Quantitative Ultrasound measurements		
	AD-SoS	***UBPI***	***BTT***
Adipose Indices			
Total body % Fat	-0.01	-0.32	**-0.35**
Fat mass / Height^2 *(Kg/m^2)*	0.04	-0.28	-0.27
Android / Gynoid ratio	0.09	**0.42**	**0.51**
%Fat trunk / %Fat legs	-0.02	0.25	0.27
Trunk/Legs fat mass ratio	0.18	0.25	0.24
Lean Indices			
Lean/Height^2 *(Kg/m^2)*	0.16	0.07	0.29
Appen. Lean/Height^2 *(Kg/m^2)*	0.03	0.03	0.22

Statistically significant values of "r" ($p<0.05$) are shown in bold. AD-SoS= Amplitude Dependent Speed of Sound; UBPI= Ultrasound Bone Profiler Index; BTT= Bone Transmission Time.

Table 5. Correlation coefficients (r) between DXA derived measurements.

	Lumbar spine BMD	***Femoral neck BMD***	***Trabecular Bone Score***
Adipose Indices			
Total body % Fat	-0.17	-0.35	**-0.45**
Fat mass / Height^2 *(Kg/m^2)*	-0.07	-0.31	**-0.42**
Android / Gynoid ratio	0.19	0.23	0.29
%Fat trunk / %Fat legs	0.17	0.16	0.11
Trunk/Legs fat mass ratio	-0.001	-0.08	-0.11
Lean Indices			
Lean/Height^2 *(Kg/m^2)*	**0.41**	0.09	0.004
Appen. Lean/Height^2 *(Kg/m^2)*	**0.41**	0.19	0.19

Statistically significant values of "r" ($p<0.05$) are shown in bold.