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Increased bone inflammation in type 2 diabetes and obesity correlates with Wnt signaling downregulation and reduced bone strength

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eLife Assessment

This is a **convincing** paper that addresses topics **important** in our understanding of how inflammatory markers are modulated in both obesity and type 2 diabetes and their effects on Wnt signaling mediators in human bone. There are changes in bone at the tissue level in these 2 common metabolic disorders that ultimately lead to compromised bone strength. These data will be critical to our understanding of the pathophysiology of skeletal fragility in obesity and diabetes.

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Abstract

Type 2 diabetes (T2D) and obesity (OB) are associated with chronic low-grade inflammation and increased fracture risk. *In vitro* studies showed that inflammation induces bone erosion and inhibits bone formation by increasing Wnt canonical pathway inhibitors. However, the

impact of inflammation on Wnt pathway regulation and bone quality in T2D and OB remains unclear. To this end, we studied 63 postmenopausal women (age >65 years) undergoing hip replacement for osteoarthritis. Among these women, 19 had T2D and OB (HbA1c 6.8±0.79%; BMI 29.9±5.2 kg/m2), 17 had OB but they were normoglycemic (BMI 32.5±5.4 kg/m2), and 27 served as controls (BMI 23.1±5.5 kg/m2). Serum inflammatory cytokines by automated immunoassay (ELLA), revealed higher TNF-α (p=0.0084) and lower adiponectin (p=0.0402) in T2D, and higher IL-6 (p=0.0003) levels in OB vs controls. Gene expression analysis of trabecular bone showed increased TNF-α (p=0.0019) and SFRP5 (p=0.0084) in T2D vs controls. IL-10 was lower in both T2D (p=0.0285), and OB (p=0.0324), while adiponectin (ADIPOQ) was only lower in T2D (p=0.0041) vs controls. Interestingly, the Wnt inhibitor SOST was higher in T2D (p<0.0001) and OB (p<0.0001) vs controls. Conversely, WNT10B mRNA levels were lower in T2D (p=0.0071) and in OB (p=0.0196) vs controls, while LEF-1 were only lower in T2D (p=0.0009). WNT5A (p=0.0025) and GSK3β (p=0.0003) mRNA levels were higher only T2D vs controls. Importantly, TNF-α mRNA levels positively correlated with SOST (r=0.5121, p=0.0002), WNT5A (r=0.3227, p=0.0396) and GSK3β (r=0.3789, p=0.0146) mRNA levels, but negatively correlated with WNT10B (r=0.3844, p=0.0188) and LEF-1(r=-0.3310, p=0.0322) mRNA levels. Conversely, IL-10 was negatively correlated with SOST mRNA levels (r=0.3100, p=0.0457). ADIPOQ was negatively correlated with SOST (r=-0.3864, p=0.0105) and WNT5A (r=-0.3025, p=0.0515) mRNA levels. Moreover, SFRP5 was negatively correlated with LEF-1 mRNA levels (r=0.3991, p=0.0131). Finally, serum levels of TNF-α (r=-0.3473, p=0.0352) and IL-6 (r=-0.3777, p=0.0302) negatively correlated with Young's Modulus, an index of bone strength. These findings suggest that increased inflammation in bone of subjects with T2D and obesity is negatively associated with Wnt pathway and bone strength, shedding light on pathophysiology of bone impairment in T2D and obesity.

Introduction

Type 2 diabetes (T2D) and obesity (OB) are metabolic diseases characterized by systemic chronic inflammation [1]. In particular, serum levels of pro-inflammatory cytokines IL-6 and TNF-α are higher in T2D and OB [2,3]. This chronic inflammatory state contributes to tissue inflammation in insulin target tissues, and it is associated to long-term complications, including bone fragility [4]. Subjects with T2D or OB have an increased risk for fractures despite normal or higher bone mineral density (BMD), suggesting a bone quality impairment. Furthermore, subjects with elevated pro-inflammatory cytokines have been linked to an increased risk of fracture [5–7]. However, data on the direct effect of inflammation on bone fragility in subjects with T2D or OB are lacking. *In vitro* studies showed that IL-6 is mainly involved in promoting osteoclastogenesis [8]. Moreover, IL-6 contributed to bone loss *in vitro*, affecting bone marrow mesenchymal stem cells (BMCs) differentiation and cellular senescence in a model of high fat diet-induced obesity [9]. On the other hand, blocking TNF-α in bone-lining forming cells increased cell number and promoted bone formation in a model of T2D [10]. Consistently, Sun M. et al. demonstrated that subjects with T2D and fractures had higher TNF-α levels [3]. Previous data showed that TNF-α promoted bone erosion and inhibited bone formation by increasing the main inhibitors of Wnt canonical pathway [11,12]. Wnt pathway is one of the main regulators of bone metabolism. We previously showed that canonical Wnt pathway is downregulated in T2D, and it is associated with reduced bone formation markers [13] and reduced bone strength [14]. Thus, we hypothesized that increased inflammation may affect bone quality by Wnt canonical pathway in bone of postmenopausal women with T2D or obesity. Results confirmed that subjects with T2D and OB have increased bone inflammation associated with Wnt beta/catenin pathway downregulation and reduced bone strength.

Results

Subject characteristics

Clinical characteristics of study subjects are reported in **Table 1**. Age and Menopausal age were not different between groups while BMI was higher in T2D and OB compared to controls (T2D: 29.98±5.77 kg/m^2; OB: 32.84±2.94 kg/m^2; CTRL: 23.11±2.24 kg/m^2, p<0.0001). As expected, fasting glucose was significantly higher in T2D compared to either OB or controls [T2D: 121.8±20.59 mg/dl; OB: 101.7±10.88 mg/dl; CTRL: 94.98±8.45 mg/dl, p<0.0001). T2D subjects had a mean HbA1c of 6.87±0.79% and a mean disease duration of 13.75±9.54 years. Diabetes medications included monotherapy with metformin (n=15) and combination therapy with metformin plus insulin and glinide (n=3). There were no differences in serum calcium, eGFR (CKD-EPI equation) and serum blood urea nitrogen, as well as in cholesterol values and triglycerides.

Adipose tissue composition and bone mass

All the parameters related to adipose tissue composition and bone mass are reported in **Table 2**. Trunk total mass was significantly higher in subjects with obesity (p<0.0001) and T2D (p=0.001) compared to controls. Also, trunk fat mass was higher in OB (p=0.003) and T2D (p=0.016) compared to controls. Consistently, percent trunk fat was higher in subjects with obesity (p=0.001) and T2D (p=0.030) as compared to control subjects. Analysis of fat distribution highlighted that OB, but not T2D, had both a higher percentage of android fat (p<0.0001) and of gynoid fat (p=0.022) as compared to controls. Finally, levels of VAT mass and VAT volume were significantly greater in subjects with obesity (p<0.0001, respectively) and T2D (p=0.0002, respectively) compared to controls.

Total body bone mineral density (BMD) and T-score were not different between groups. Lumbar bone mineral density was higher in OB compared to controls (p=0.004) but not in T2D compared to controls. Lumbar T-score was also increased only in OB compared to controls (p=0.003). Femoral BMD was increased not only in OB (p=0.0005) but also in T2D (p= 0.016) compared to controls. In line, femoral T-score was higher in OB (p<0.0001) and T2D (p=0.006) compared to controls.

Bone microarchitecture and strength

Microarchitecture parameters by µCT on trabecular core samples are reported in **Table 3**. T2D, OB and control subjects had similar trabecular indices, including bone volume density (BV/TV), connectivity density, trabecular thickness, trabecular number, trabecular separation, tissue mineral density and volumetric BMD. Biomechanic analysis (**table 3**) showed a reduced Young's modulus in T2D compared to OB (p=0.0395) but not to control subjects, and a trend of reduction of Yield strength in T2D compared to OB (p=0.0552).

Circulating and bone inflammation

Circulating TNF-α levels were significantly higher in T2D (p=0.0004), but they were not higher in OB (p=0.1108) compared to control subjects (**Fig. 1A**). IL-6 levels were higher in both T2D (p=0.0451) and OB (p=0.0002) compared to controls (**Fig. 1B**). Adiponectin levels were lower only in T2D compared to OB (p=0.0402), but not in the other groups (**Fig. 1C**). Of note, circulating TNF-α and IL-6 were strongly positively correlated with VAT mass (r=0.3651, p=0.0310 and r=0.7139, p<0.0001, respectively), as reported in **Figure 1-Supplementary figure 1**.

	T2D (A) n=19	OB (B) n=17	CTRL (C) N=27	P value	Multiple Comparisons
Age (years)	74.90±7.22	71.84±9.63	71.92±8.36	0.375	NS
BMI (kg/m^2)	29.98±5.77	32.84±2.93	23.11±2.24	<0.0001	A-B=0.010 A-C<0.0001 B-C<0.0001
Menopausal age (years)	48.65±5.18	49.03±4.78	51.13±3.47	0.098	NS
Fasting glucose levels (mg/dl)	121.8±20.59	101.7±10.88	94.98±8.45	<0.0001	A-B<0.0001 A-C<0.0001 B-C=0.0623
HbA1c (%)	6.88±0.79	-	-	-	-
Disease duration	13.75±9.54	-	-	-	-
Serum calcium (mg/dl)	9.29± 0.43	9.20±0.44	9.16±0.34	0.659	NS
eGFR(mL/min/1.73 m^2)					
Serum blood urea nitrogen (mg/dl)	45.40±14.23	45.07±13.36	38.52±9.10	0.076	NS
Total cholesterol (mg/dl)	183.7±22.05	213.3±27.69	221.3±53.90	0.393	NS
HDL cholesterol (mg/dl)	48.00±6.24	63.46±18.16	65.42±12.27	0.170	NS
LDL cholesterol (mg/dl)	107.3±28.92	126.5±26.77	130.3±48.87	0.672	NS
Triglycerides (mg/dl)	165.0±35.16	123.0±48.45	111.3±50.32	0.208	NS

Table 1.

Clinical characteristics of the study subjects Results were analyzed using unpaired Ordinary One-Way ANOVA and Tukey's Multiple Comparisons test. Results are presented as mean ± standard deviation.

	T2D (A) n=19	OB (B) n=17	CTRL (C) N=27	P value	Multiple Comparisons
Trunk total mass (gr)	39718±5094	42018±5861	31431±6753	<0.0001	A-C=0.001 B-C<0.0001
Trunk fat mass (gr)	19984±3637	20219±7444	14232±4870	0.0015	A-C=0.016 B-C=0.003
Trunk fat percentage (%)	50.08±4.065	51.33±6.833	44.22±6.465	0.001	A-C=0.030 B-C=0.001
Android fat percentage (%)	49.84±3.076	54.18±5.252	45.34±7.379	0.0001	B-C<0.0001
Gynoid fat percentage (%)	48.52±5.356	50.62±4.704	46.17±5.349	0.028	B-C=0.022
VAT mass (gr)	1170±284.8	1172±370.0	675.2±244.3	<0.0001	A-C=0.0002 B-C<0.0001
VAT volume (cm^3)	1265±307.8	1267±400.1	729.8±264.3	<0.0001	A-C=0.0002 B-C<0.0001
BMD total body (g/cm^2)	1.255±0.134	1.259±0.108	1.228±0.123	0.679	NS
T score total body	1.708±1.184	1.905±1.475	1.556±1.546	0.709	NS
BMD Lumbar L1+L4 (g/cm^2)	0.9512±0.120	1.028±0.156	0.873±0.143	0.005	B-C=0.004
T score Lumbar	-0.866±1.094	-0.166±1.422	-1.581±1.305	0.005	B-C=0.003
BMD Femoral (g/cm^2)	0.856±0.125	0.873±0.119	0.727±0.070	0.0004	A-C=0.016 B-C=0.0005
T score Femoral	-0.685±1.042	-0.550±0.976	-1.854±0.543	<0.0001	A-C=0.006 B-C=0.0001

Table 2.

Adipose tissue composition and bone mass by DXA Results were analyzed using unpaired Ordinary One-Way ANOVA and Tukey's Multiple Comparisons test. Results are presented as mean ± standard deviation.

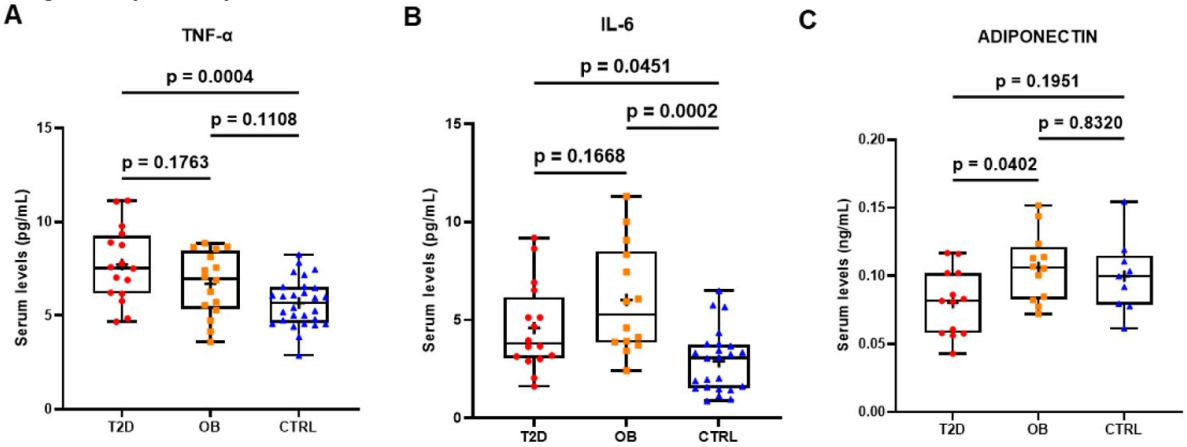
Table 3.

Microarchitecture and mechanical parameters of trabecular bone of the study subjects, Results were analyzed using unpaired Ordinary One-Way ANOVA and Tukey's Multiple Comparisons test. Results are presented as mean ± standard deviation.

	T2D (A) n=19	OB (B) n=17	CTRL (C) N=18	P value	Multiple Comparisons
Microcomputed tomography					
BV/TV	0.240±0.135	0.236±0.096	0.291±0.092	0.396	NS
Connectivity density(1/mm^3)	6.992±4.251	6.754±2.913	6.748±1.839	0.978	NS
Trabecular number (1/mm)	1.484±0.501	1.542±0.197	1.520±0.266	0.926	NS
Trabecular thickness (mm)	0.203±0.054	0.177±0.067	0.211±0.053	0.367	NS
Trabecular separation (mm)	0.709±0.197	0.585±0.114	0.582±0.090	0.074	NS
Volumetric BMD (mgHA/cm^3)	220.4±100.2	224.1±78.07	263.5±68.33	0.381	NS
Tissue mineral density (mgHA/cm^3)	689.4±32.80	680.7±29.59	690.7±29.80	0.681	NS
Biomechanical properties					
Young's Modulus (MPa)	23.68±10.82	69.28±59.23	50.75±46.06	0.050	A-B=0.039
Ultimate strength (MPa)	3.632±2.178	5.607±3.344	5.199±3.021	0.256	NS
Yield strength (MPa)	2.410±1.160	4.849±2.777	4.591±2.442	0.049	A-B=0.0559

Figure 1.

Circulating inflammatory cytokines of subjects with T2D, OB and controls. Serum (A) Tumor Necrosis Factor-alpha (TNF-α), (B) Interleukin-6 (IL-6), and (C) Adiponectin. The unit of TNF-α and IL-6 concentrations was pg/mL, while adiponectin concentration was ng/mL. The box plots show the medians (middle line) and first and third quartiles (boxes), and the whiskers show the minimum and maximum. Medians and interquartile ranges, differences between groups were analyzed using Ordinary One-Way ANOVA.



Analysis of gene expression in the femoral head after surgery showed increased TNF-α mRNA levels either in T2D compared to controls (p<0.0001) or in T2D compared to OB (p=0.0161) (**Fig. 2A**). However, gene expression levels of IL-6 were not different between groups (**Fig. 2B**), as for IL-8 mRNA levels (**Fig. 2C**) IL-10 gene expression levels were lower in both T2D (p=0.0285), and OB (p=0.0324) compared to controls (**Fig. 2D**). Adiponectin (ADIPOQ) mRNA levels were lower in subjects with T2D (p=0.0041) compared to controls (**Fig. 2E**). On the other hand, SFRP5 mRNA levels were only higher in T2D (p=0.0084) compared to control subjects (**Fig. 2F**).

Wnt signaling pathway in bone

The canonical Wnt inhibitor SOST mRNA levels were higher in T2D (p<0.0001) and in OB (p<0.0001) compared to controls (**Fig.3A**). Interestingly, SOST mRNA levels were positively correlated with fasting blood glucose levels in OB (**Figure 3-Supplementary figure 1**). In line, gene expression of the canonical ligand WNT10B was lower in T2D (p=0.0071) and in OB (p=0.0196) vs controls (**Fig. 3B**). Also, gene expression of LEF-1, the Wnt/β-catenin transcription factor, was lower in T2D (p<0.0001) and in OB (p=0.0479) compared to controls (**Fig. 3C**). Gene expression of non-canonical WNT5A was higher only in T2D (p=0.0025) but it was not different in OB compared to controls (**Fig. 3D**). Finally, Gene expression of GSK3β was higher either in T2D compared to OB (p=0.0069) or T2D vs controls (p=0.0003) (**Fig. 3E**).

Correlation analysis of inflammatory and Wnt target genes

Correlation analysis of inflammatory and Wnt target genes are reported in **figure 4** (A-I). TNF-α mRNA levels were positively correlated with SOST (r=0.5121, p=0.0002), WNT5A (r=0.3227, p=0.0396) and GSK3β (r=0.3789, p=0.0146) mRNA levels (**Fig. 4A-C**). Conversely, TNF-α was negatively correlated with WNT10B (r=-0.3844, p=0.0188) and LEF-1 (r=-0.3310, p=0.0322) mRNA levels (**Fig. 4D,E**). IL-10 was only negatively correlated with SOST (r=-0.3100, p=0.0457) (**Fig. 4F**). ADIPOQ mRNA levels were negatively correlated with SOST (r=-0.3864, p=0.0105) and with WNT5A mRNA levels (r=-0.3025, p=0.0515) (**Fig. 4G,H**). Moreover, SFRP5 mRNA levels negatively correlated with LEF-1 (r=-0.3991, p=0.0131) mRNA levels (**Fig. 4I**).

Correlation analysis of inflammatory cytokines and bone strength

Serum levels of TNF-α (r=-0.3473, p=0.0352) and IL-6 (r=-0.3777, p=0.0302) negatively correlated with Young's Modulus (**Fig. 5A-B**). There was no difference between circulating inflammatory markers and the other biomechanical parameters.

Discussion

In this study we showed increased circulating and bone inflammation in postmenopausal women with T2D and obesity. Specifically, we found increased TNF-α mRNA levels in bone of subjects with T2D while the anti-inflammatory cytokine IL-10 was decreased in both subjects with T2D and obesity. Importantly, increased mRNA levels of inflammatory cytokines were associated with downregulation of Wnt pathway related genes. Moreover, serum TNF-α was increased in T2D compared to the other groups and it was negatively associated with bone strength.

The increased TNF-α serum levels in subjects with T2D observed in our study are consistent with the study of Sum et al. [3] that reported increased TNF-α and other chemokines in T2D patients with bone fracture. There are evidence showing that inflammation may affect bone health. *In vitro* studies showed that TNF-α is involved in the regulation of bone resorption in inflammatory conditions either directly or by increasing RANKL and M-CSF expressions by osteoblasts, stromal cells and even osteocytes [15]. Moreover, blocking TNF-α in bone-lining forming cells increased

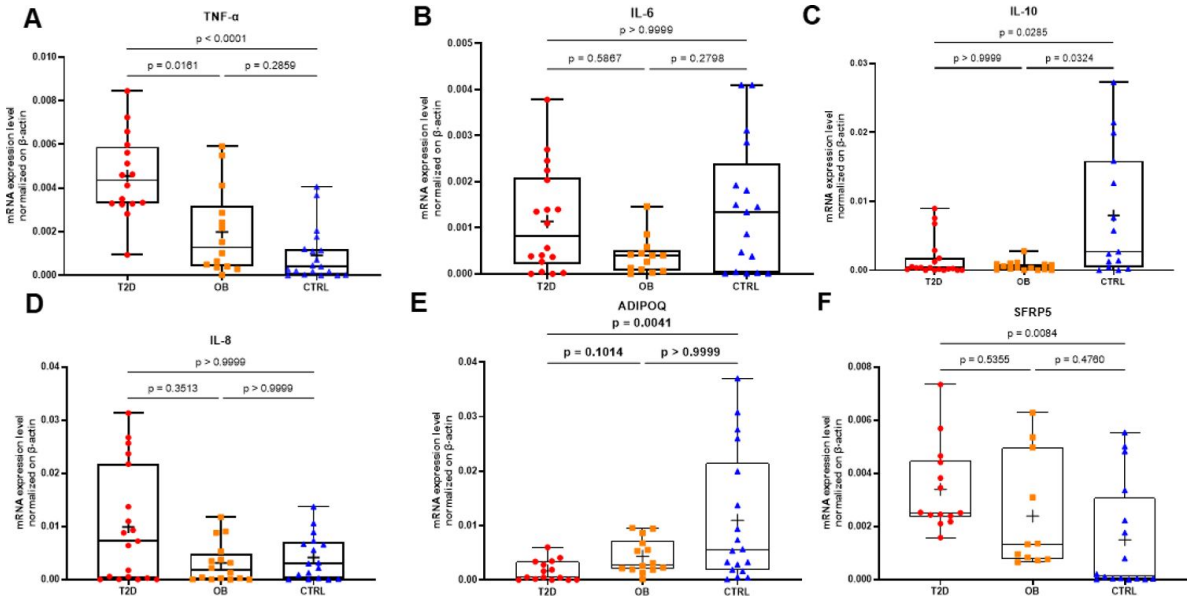


Figure 2.

Gene expression analysis of inflammatory markers in trabecular bone samples of subjects with T2D, OB and controls.

mRNA levels of (A) Tumor Necrosis Factor-alpha (TNF-α), (B) Interleukin-6 (IL-6), (C) Interleukin-10 (IL-10), (D) Interleukin-8 (IL-8), (E) Adiponectin (ADIPOQ), and (F) secreted frizzled-related protein 5 (SFRP5). Data are expressed as fold changes over beta-actin. Medians and interquartile ranges, differences between groups were analyzed using non-parametric One-Way ANOVA (Kruskal-Wallis test).

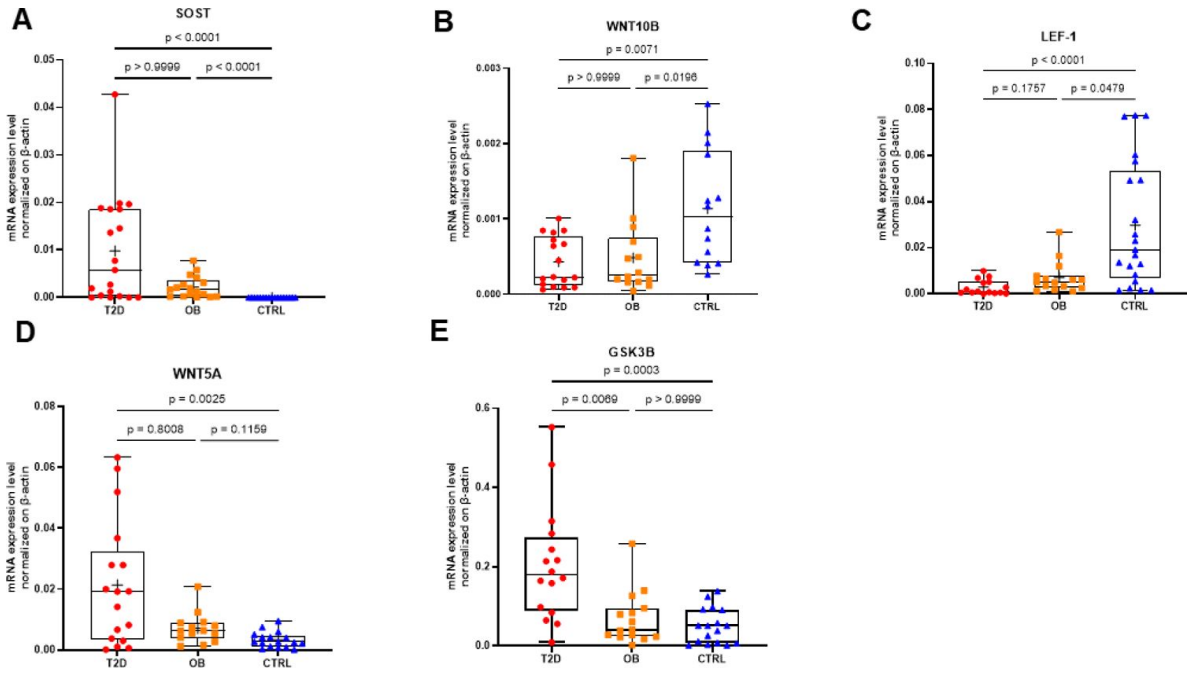


Figure 3.

Gene expression analysis of Wnt signaling pathway in trabecular bone samples of subjects with T2D, OB and controls.

mRNA levels of (A) Sclerostin (SOST) (B) Wnt family member 10B (WNT10B), (C) T-cell factor/ lymphoid enhancer factor 1 (LEF-1), (D) Wnt family member 5A (WNT5A), and (E) glycogen synthase kinase 3 beta (GSK3β). Data are expressed as fold changes over beta-actin. Medians and interquartile ranges, differences between groups were analyzed using non-parametric One-Way ANOVA (Kruskal-Wallis test).

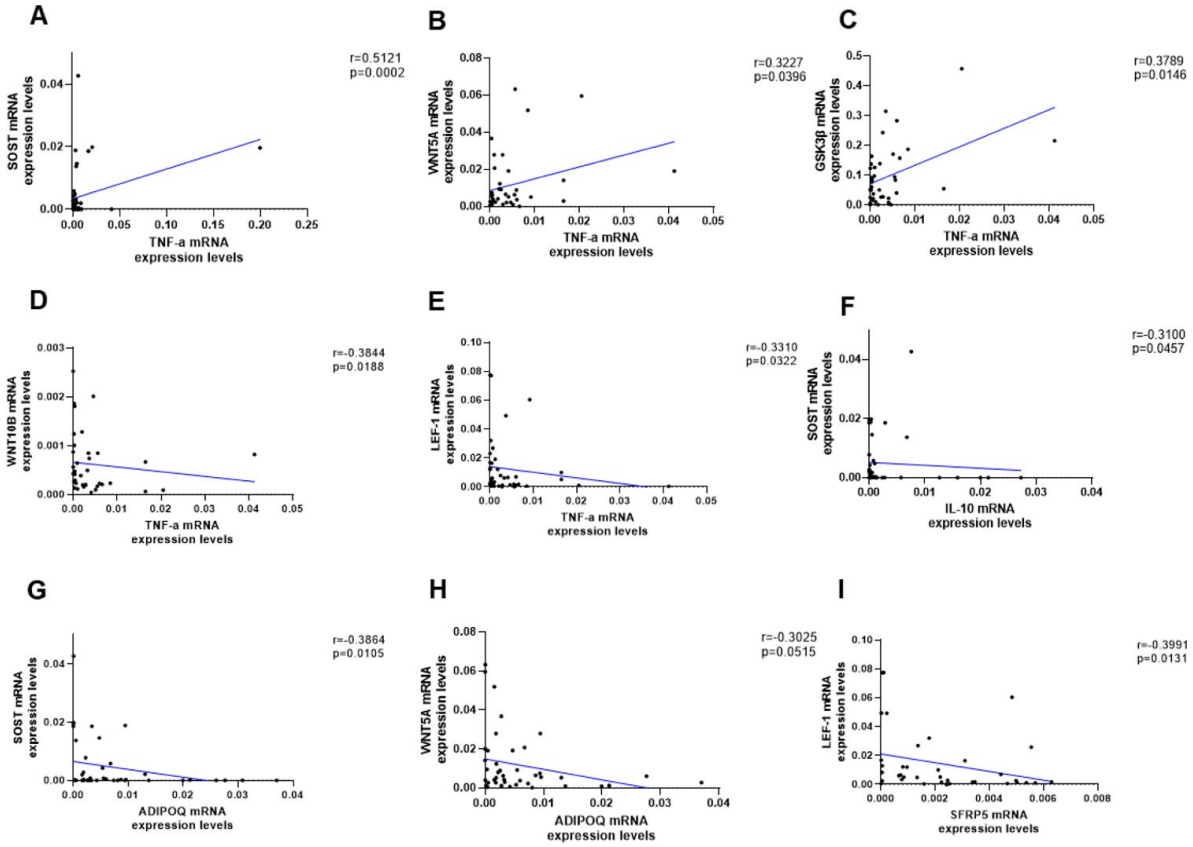


Figure 4.

Correlation analysis of inflammatory and Wnt target genes in all study subjects.

Correlations between Tumor Necrosis Factor-alpha (TNF-α) and (A) sclerostin (SOST), (B) Wnt family member 5A (WNT5A), (C) glycogen synthase kinase 3 beta (GSK3β), (D) Wnt family member 10B (WNT10B), and (E) T-cell factor/ lymphoid enhancer factor 1 (LEF-1). (F) Correlation between Interleukin-10 (IL-10) and sclerostin (SOST). Correlation between Adiponectin (ADIPOQ) and (G) sclerostin (SOST), (H) Wnt family member 5A (WNT5A). (I) Correlation between secreted frizzled-related protein 5 (SFRP5) and T-cell factor/ lymphoid enhancer factor 1 (LEF-1). Data were analyzed using nonparametric Spearman correlation analysis and r represents the correlation coefficient.

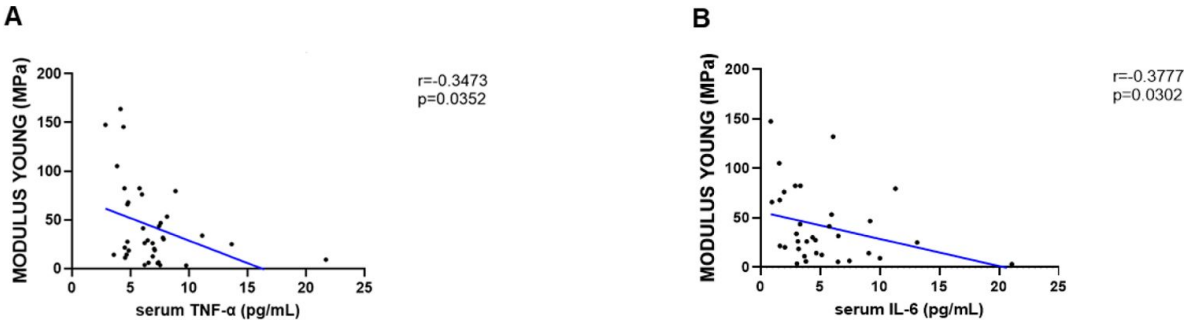


Figure 5.

Correlation analysis of circulating inflammatory cytokines and Young's Modulus in all study subjects.

Correlations between (A) Tumor Necrosis Factor-alpha (TNF-α) and Young's Modulus, and (B) Interleukin-6 (IL-6) and Young's Modulus. Data were analyzed using nonparametric Spearman correlation analysis and r represents the correlation coefficient.

cell number and enhanced bone formation in a model of T2D [10]. In line, we found that both serum and bone TNF-α were increased in T2D, but not in subjects with obesity. Interestingly, serum TNF-α was negatively correlated with Young's Modulus, suggesting that increased TNF-α reduces bone strength in subjects with T2D.

Although we confirmed that circulating IL-6 was higher in subject with obesity and it was positively correlated with VAT, we found no difference of IL-6 gene expression levels in bone tissue of either T2D or subjects with obesity. There is only evidence that IL-6 contributed to bone loss *in vitro*, affecting BMCs differentiation and cellular senescence in a model of high fat diet-induced obesity [9]. However, there is no data supporting the effect of inflammation in bone of subjects with obesity. In line with IL-6, gene expression of pro-inflammatory IL-8 was not altered either in bone of subjects with T2D or obesity. There is no evidence about IL-8 role in bone of either T2D or obesity [8].

On the other hand, we found that the anti-inflammatory IL-10 mRNA levels were decreased in subjects with T2D and obesity. Previous data showed that serum levels of IL-10 were substantially lower in osteoporosis patients [16] and in T2D [17] than in healthy individuals, although to our knowledge, the role of IL-10 on bone of T2D or obesity has not been studied. As expected, both serum and mRNA bone adiponectin levels were lower in T2D [18,19]. *In vitro* studies indicated that adiponectin promoted osteoblastogenesis, whilst simultaneously inhibiting osteoclastogenesis [20–22] and adiponectin knockout mice have increased bone mass and osteoblast numbers [23].

Moreover, serum adiponectin in postmenopausal women was found inversely associated with bone mass [24]. However, there are no data about adiponectin in bone of subjects with T2D or obesity. We also showed that subjects with T2D had increased SFRP5 bone mRNA levels compared to controls. Data reported that SFRP5, a negative regulator of Wnt canonical signaling pathway, was overexpressed in osteoporosis and was also associated to downregulated bone formation markers [25]. Our data are in line with these findings, supporting that T2D is characterized by low bone formation and downregulated Wnt canonical signaling.

Previous studies showed that inflammation may downregulate Wnt signaling pathway [26], the main regulator of bone metabolism. Consistent with our previous findings [14], we observed that the expression of the Wnt inhibitor SOST was not only higher in bone of T2D but also in subjects with obesity compared to controls. Even though sclerostin is increased during weight loss [27], there are other data showing that serum sclerostin was negatively associated to insulin sensitivity in women with obesity [28]. Conversely, our data showed that SOST mRNA levels were positively correlated with fasting blood glucose in subjects with obesity suggesting that glucose impairment may be a determinant for higher sclerostin levels. Consistent with increased SOST mRNA levels and in line with our previous findings on Wnt signaling regulation in bone of T2D [14], the expression of the Wnt ligand WNT10B and the transcription factor LEF-1 were downregulated in both subjects with T2D and obesity. Also, *in vitro* studies showed that TNF-α induced the phosphorylation of GSK3β, the deactivated form of GSK3β, which increased nuclear β-catenin and Lef-1 resulting in downregulated Wnt signaling and reduced bone formation [26].Our data confirmed that GSK3βB is increased in subjects with T2D. This is in line with our previous findings in which GSK3β was associated with reduced bone formation and strength in T2D [14].

Furthermore, it is shown that Wnt5a promotes inflammation and insulin resistance in adipose tissue of human and animal models [29,30] and high circulating levels of Wnt5a are detected in subjects with T2D [31] and obesity [32]. Other studies showed that Wnt5a inhibits Wnt3a protein by downregulating beta/catenin-induced reporter gene expression [33]. In line, we observed that gene expression of WNT5A was increased in bone of T2D. This study has some limitations. It is a cross-sectional design with a relatively small number of enrolled subjects.

The main strength of the study is that we investigated for the first time the effect of inflammation on human bone samples measuring several pro and anti-inflammatory genes. Moreover, we found the association between inflammation and bone strength by the regulation of Wnt signaling pathway in subjects with T2D and obesity compared to a control population.

In conclusion, our study showed that there is increased inflammation in bone of subjects with T2D and obesity and it is associated with the downregulation of Wnt signaling and reduced bone strength. These results shed light on the pathophysiology of bone impairment in T2D and obesity and may help understanding the mechanisms of bone fragility in metabolic diseases.

Material and methods

Study subjects

We enrolled for this study 63 postmenopausal women (19 with T2D, 17 with obesity and 27 non-diabetic normal weight controls) undergoing hip replacement surgery for osteoarthritis, consecutively screened to participate in this study between 2018 and 2022. Diabetes status was confirmed by the diabetologist. Participants were diagnosed with type 2 diabetes when they had fasting plasma glucose (FPG) ≥126 mg/dl or 2-h plasma glucose (2-h PG) ≥200 mg/dl during a 75-g oral glucose tolerance test (OGTT); or hemoglobin A1c (HbA1c) ≥6.5% in accordance with the American Diabetes Association diagnostic criteria [34]. Obesity was confirmed based on the BMI ≥30 kg/m^2, in accordance with the World Health Organization diagnostic criteria [35]. Eligible participants were ≥60 years of age. Exclusion criteria were any diseases affecting bone, any concomitant inflammatory disease or malignancy. Additionally, individuals treated with medications affecting bone metabolism such as estrogen, raloxifene, tamoxifen, bisphosphonates, teriparatide, denosumab, thiazolidinediones, glucocorticoids, anabolic steroids, and phenytoin, and those with hypercalcemia or hypocalcemia, hepatic or renal disorder, hypercortisolism, current alcohol or tobacco use were excluded. The study was approved by the Ethics Committee of Campus Bio-Medico University of Rome (Prot..42/14 PT_ComEt CBM) and all participants provided written informed consent. All procedures were conducted in accordance with the Declaration of Helsinki.

Body Composition/Anthropometric measurements

Body scans were conducted with Lunar ProdigyTM (GE Healthcare©, USA) DXA scanner. Body composition parameters were analyzed using en-CORETM software (version 17, 2016), and VAT was measured with the CoreScanTM (GE Healthcare©, USA). Quality control and calibration of the model were performed each day, following the instruction protocol provided by the manufacturer. For this analysis, the variables of interest derived from the DXA dataset are BMD total body (g/cm^2), T score total body, BMD Lumbar L1+L4 (g/cm^2), T score femoral, trunk fat mass (gr), trunk total mass (gr), and defined anatomical regions (android, and gynoid % fat) and visceral adipose tissue (VAT mass, g and VAT volume, cm^3). The body mass index (BMI) was calculated as weight/(height)^2 (kg/m^2).

Processing of blood Samples

Venous whole blood was collected the day before the surgery in all participants after completing a fasting period of at least 8 hours, using tubes containing clot activator (BD vacutainer®, NJ, USA). Samples were kept at room temperature to allow the blood to clot, and they were centrifuged at 1500 g for 10 minutes for serum isolation. Serum was then transferred to a clean tube and stored at −80°C for further analysis.

Processing of bone samples

Femoral head specimens were obtained during hip replacement surgery. Briefly, trabecular bone specimens were collected fresh and washed multiple times in sterile PBS until the supernatant was clear of blood, as previously described [13]. Trabecular samples were then aliquoted and stored at −80°C for further analysis.

ELISA and ELLA Microfluidic Immunoassay

Adiponectin was quantified in serum using ELISA immunoassay (R&D system, MN, USA) according to the manufacturer instructions. TNF-α and IL-6 were measured in serum by using Simple Plex assays run on the ELLA microfluidic immunoassay system (ProteinSimple, San Jose, CA). 25 µl of serum was diluted at a 1:1 ratio with sample diluent, and 50 µl the solution was added to each sample inlet on the ELLA cartridge, according to manufacturer's instruction. Sample results were reported using Simple Plex Runner v.3.7.2.0 (ProteinSimple).

RNA extraction and gene expression by RT-PCR

Total RNA from trabecular bone samples was extracted using TRIzol (Invitrogen) following the manufacturer's instructions. The concentration and purity of the extracted RNA were assessed spectrophotometrically (TECAN, InfiniteM200PRO), and only samples with 260/280 absorbance ratio between 1.8 and 2 were used for reverse transcription using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA) according to the manufacturer's recommendations. Transcription products were amplified using TaqMan real-time PCR (Applied Biosystems, Carlsbad, CA) and a standard protocol (95°C for 10 minutes; 40 cycles of 95°C for 15 seconds and 60°C for 1 minute; followed by 95°C for 15 seconds, 60°C for 15 seconds, and 95°C for 15 seconds). β-Actin expression was used as an internal control (housekeeping gene). Relative expression levels of Tumor necrosis factor alpha (TNF-α), Interleukin 6 (IL-6), Adiponectin (ADIPOQ), Interleukin 8 (IL-8), Interleukin 10 (IL-10), secreted frizzled-related protein 5 (SFRP5), Sclerostin (SOST), Wnt ligands (WNT5A and WNT10B), T-cell factor/ lymphoid enhancer factor 1 (LEF-1), and glycogen synthase kinase 3 beta (GSK3B), were calculated using the 2-ΔCt method.

Micro-computed tomography (µCT) and bone compression tests

We used cylindrical bone specimens of trabecular core (with a diameter of 10 mm and a length of 20 mm) from 19 T2D with obesity, 17 OB non-diabetic and 18 control subjects to measure bone micro-architecture and bone mechanical parameters (Young's modulus, ultimate strength and yield strength), as previously described [13].

Statistical analysis

Data were analyzed using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA). Patients' characteristics were described using means and standard deviations (SD) or medians and 25th-75th percentiles, as appropriate, and percentages. Group data are presented in boxplots with median and interquartile range (IQR); whiskers represent maximum and minimum values. We assessed data for normality and Mann-Whitney test was used to compare variables between groups. Data were analyzed using nonparametric Spearman correlation analysis and the correlation coefficients (r) were used to assess the relationship between variables. We used Grubbs' Test to assess and exclude outliers.

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Supplementary figure legends

Figure 1-Supplementary figure 1.

Correlation analysis of circulating inflammatory cytokines and Visceral adipose tissue (VAT) mass in all study subjects.

Correlations between (A) Tumor Necrosis Factor-alpha (TNF-α) and VAT mass, and (B) Interleukin-6 (IL-6) and VAT mass. Data were analyzed using Pearson correlation analysis and r represents the correlation coefficient.

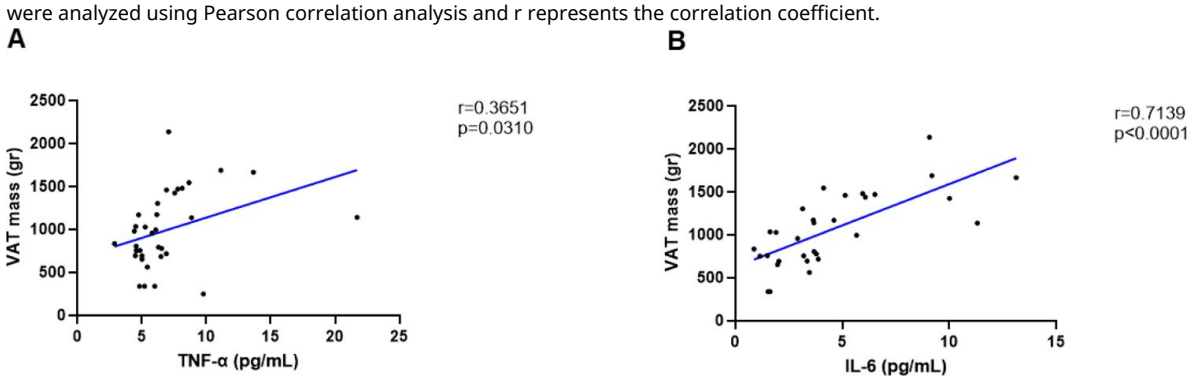
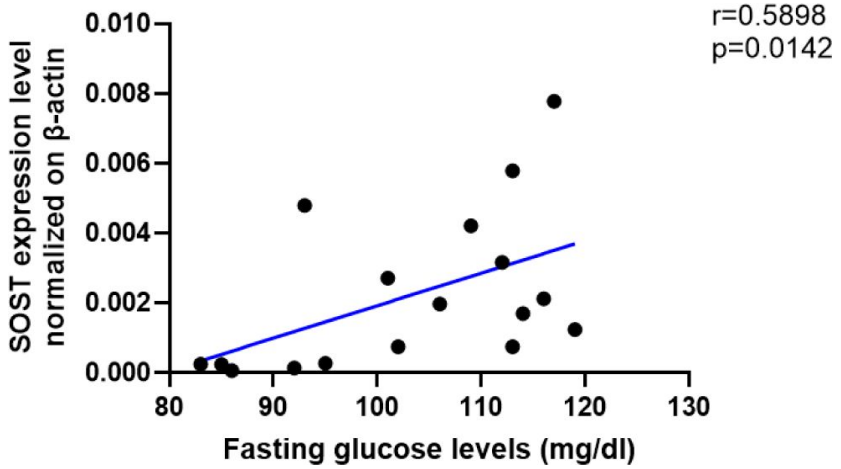


Figure 3-Supplementary figure 1.

Correlation analysis of SOST mRNA levels in bone and fasting blood glucose in subjects with obesity (OB). Data were analyzed using nonparametric Spearman correlation analysis and r represents the correlation coefficient.



References

[1] Rohm T V, Meier DT, Olefsky JM, Donath MY (2022) **Inflammation in obesity, diabetes, and related disorders** *Immunity* **55**:31–55 https://doi.org/10.1016/j.immuni.2021.12.013

[2] Park HS, Park JY, Yu R (2005) **Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-alpha and IL-6** *Diabetes Res Clin Pract* **69**:29–35 https://doi.org/10.1016/j.diabres.2004.11.007

[3] Sun M, Yang J, Wang J, Hao T, Jiang D, Bao G, et al. (2016) **TNF-α is upregulated in T2DM patients with fracture and promotes the apoptosis of osteoblast cells in vitro in the presence of high glucose** *Cytokine* **80**:35–42 https://doi.org/10.1016/j.cyto.2016.01.011

[4] Bathina S, Armamento-Villareal R (2023) **The complex pathophysiology of bone fragility in obesity and type 2 diabetes mellitus: therapeutic targets to promote osteogenesis** *Front Endocrinol (Lausanne* **14** https://doi.org/10.3389/fendo.2023.1168687

[5] Cauley JA, Barbour KE, Harrison SL, Cloonan YK, Danielson ME, Ensrud KE, et al. (2016) **Inflammatory Markers and the Risk of Hip and Vertebral Fractures in Men: the Osteoporotic Fractures in Men (MrOS)** *J Bone Miner Res* **31**:2129–38 https://doi.org/10.1002/jbmr.2905

[6] Barbour KE, Boudreau R, Danielson ME, Youk AO, Wactawski-Wende J, Greep NC, et al. (2012) **Inflammatory markers and the risk of hip fracture: the Women's Health Initiative** *J Bone Miner Res* **27**:1167–76 https://doi.org/10.1002/jbmr.1559

[7] Barbour KE, Lui L-Y, Ensrud KE, Hillier TA, LeBlanc ES, Ing SW, et al. (2014) **Inflammatory markers and risk of hip fracture in older white women: the study of osteoporotic fractures** *J Bone Miner Res* **29**:2057–64 https://doi.org/10.1002/jbmr.2245

[8] Xu J, Yu L, Liu F, Wan L, Deng Z (2023) **The effect of cytokines on osteoblasts and osteoclasts in bone remodeling in osteoporosis: a review** *Front Immunol* **14** https://doi.org/10.3389/fimmu.2023.1222129

[9] Li Y, Lu L, Xie Y, Chen X, Tian L, Liang Y, et al. (2020) **Interleukin-6 Knockout Inhibits Senescence of Bone Mesenchymal Stem Cells in High-Fat Diet-Induced Bone Loss** *Front Endocrinol (Lausanne* **11** https://doi.org/10.3389/fendo.2020.622950

[10] Liu R, Bal HS, Desta T, Behl Y, Graves DT (2006) **Tumor necrosis factor-alpha mediates diabetes-enhanced apoptosis of matrix-producing cells and impairs diabetic healing** *Am J Pathol* **168**:757–64 https://doi.org/10.2353/ajpath.2006.050907

[11] Zhao B. (2017) **TNF and Bone Remodeling** *Curr Osteoporos Rep* **15**:126–34 https://doi.org/10.1007/s11914-017-0358-z

[12] Diarra D, Stolina M, Polzer K, Zwerina J, Ominsky MS, Dwyer D, et al. (2007) **Dickkopf-1 is a master regulator of joint remodeling** *Nat Med* **13**:156–63 https://doi.org/10.1038/nm1538

[13] Piccoli A, Cannata F, Strollo R, Pedone C, Leanza G, Russo F, et al. (2020) **Sclerostin Regulation, Microarchitecture, and Advanced Glycation End-Products in the Bone of Elderly Women With Type 2 Diabetes** *J Bone Miner Res* **35**:2415–22 https://doi.org/10.1002/jbmr.4153

[14] Leanza G, Cannata F, Faraj M, Pedone C, Viola V, Tramontana F, et al. (2024) **Bone canonical Wnt signaling is downregulated in type 2 diabetes and associates with higher advanced glycation end-products (AGEs) content and reduced bone strength** *Elife* **12** https://doi.org/10.7554/eLife.90437

[15] Marahleh A, Kitaura H, Ohori F, Kishikawa A, Ogawa S, Shen W-R, et al. (2019) **TNF-α Directly Enhances Osteocyte RANKL Expression and Promotes Osteoclast Formation** *Front Immunol* **10** https://doi.org/10.3389/fimmu.2019.02925

[16] Ma X, Zhu X, He X, Yi X, Jin A (2021) **The Wnt pathway regulator expression levels and their relationship to bone metabolism in thoracolumbar osteoporotic vertebral compression fracture patients** *Am J Transl Res* **13**:4812–8

[17] van Exel E, Gussekloo J, de Craen AJM, Frölich M, Bootsma-Van Der Wiel A, Westendorp RGJ, et al. (2002) **Low production capacity of interleukin-10 associates with the metabolic syndrome and type 2 diabetes: the Leiden 85-Plus Study** *Diabetes* **51**:1088–92 https://doi.org/10.2337/diabetes.51.4.1088

[18] Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, et al. (2000) **Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients** *Arterioscler Thromb Vasc Biol* **20**:1595–9 https://doi.org/10.1161/01.atv.20.6.1595

[19] Jalovaara K, Santaniemi M, Timonen M, Jokelainen J, Kesäniemi YA, Ukkola O, et al. (2008) **Low serum adiponectin level as a predictor of impaired glucose regulation and type 2 diabetes mellitus in a middle-aged Finnish population** *Metabolism* **57**:1130–4 https://doi.org/10.1016/j.metabol.2008.03.019

[20] Lee HW, Kim SY, Kim AY, Lee EJ, Choi J-Y, Kim JB (2009) **Adiponectin stimulates osteoblast differentiation through induction of COX2 in mesenchymal progenitor cells** *Stem Cells* **27**:2254–62 https://doi.org/10.1002/stem.144

[21] Lin YY, Chen CY, Chuang TY, Lin Y, Liu HY, Mersmann HJ, et al. (2014) **Adiponectin receptor 1 regulates bone formation and osteoblast differentiation by GSK-3β/β-catenin signaling in mice** *Bone* **64**:147–54 https://doi.org/10.1016/j.bone.2014.03.051

[22] Wu X, Huang L, Liu J (2019) **Effects of adiponectin on osteoclastogenesis from mouse bone marrow-derived monocytes** *Exp Ther Med* **17**:1228–33 https://doi.org/10.3892/etm.2018.7069

[23] Kajimura D, Lee HW, Riley KJ, Arteaga-Solis E, Ferron M, Zhou B, et al. (2013) **Adiponectin regulates bone mass via opposite central and peripheral mechanisms through FoxO1** *Cell Metab* **17**:901–15 https://doi.org/10.1016/j.cmet.2013.04.009

[24] Napoli N, Pedone C, Pozzilli P, Lauretani F, Ferrucci L, Incalzi RA (2010) **Adiponectin and bone mass density: The InCHIANTI study** *Bone* **47**:1001–5 https://doi.org/10.1016/j.bone.2010.08.010

[25] Chen H, He Y, Wu D, Dai G, Zhao C, Huang W, et al. (2017) **Bone marrow sFRP5 level is negatively associated with bone formation markers** *Osteoporos Int* **28**:1305–11 https://doi.org/10.1007/s00198-016-3873-3

[26] Kong X, Liu Y, Ye R, Zhu B, Zhu Y, Liu X, et al. (2013) **GSK3β is a checkpoint for TNF-α-mediated impaired osteogenic differentiation of mesenchymal stem cells in inflammatory microenvironments** *Biochim Biophys Acta* **1830**:5119–29 https://doi.org/10.1016/j.bbagen.2013.07.027

[27] Armamento-Villareal R, Sadler C, Napoli N, Shah K, Chode S, Sinacore DR, et al. (2012) **Weight loss in obese older adults increases serum sclerostin and impairs hip geometry but both are prevented by exercise training** *J Bone Miner Res* **27**:1215–21 https://doi.org/10.1002/jbmr.1560

[28] Aznou A, Meijer R, van Raalte D, den Heijer M, Heijboer A, de Jongh R. (2021) **Serum sclerostin is negatively associated with insulin sensitivity in obese but not lean women** *Endocr Connect* **10**:131–8 https://doi.org/10.1530/EC-20-0535

[29] Fuster JJ, Zuriaga MA, Ngo DT-M, Farb MG, Aprahamian T, Yamaguchi TP, et al. (2015) **Noncanonical Wnt signaling promotes obesity-induced adipose tissue inflammation and metabolic dysfunction independent of adipose tissue expansion** *Diabetes* **64**:1235–48 https://doi.org/10.2337/db14-1164

[30] Zuriaga MA, Fuster JJ, Farb MG, MacLauchlan S, Bretón-Romero R, Karki S, et al. (2017) **Activation of non-canonical WNT signaling in human visceral adipose tissue contributes to local and systemic inflammation** *Sci Rep* **7** https://doi.org/10.1038/s41598-017-17509-5

[31] Lu Y-C, Wang C-P, Hsu C-C, Chiu C-A, Yu T-H, Hung W-C, et al. (2013) **Circulating secreted frizzled-related protein 5 (Sfrp5) and wingless-type MMTV integration site family member 5a (Wnt5a) levels in patients with type 2 diabetes mellitus** *Diabetes Metab Res Rev* **29**:551–6 https://doi.org/10.1002/dmrr.2426

[32] Schulte DM, Müller N, Neumann K, Oberhäuser F, Faust M, Güdelhöfer H, et al. (2012) **Pro-inflammatory wnt5a and anti-inflammatory sFRP5 are differentially regulated by nutritional factors in obese human subjects** *PLoS One* **7** https://doi.org/10.1371/journal.pone.0032437

[33] Mikels AJ, Nusse R (2006) **Purified Wnt5a protein activates or inhibits beta-catenin-TCF signaling depending on receptor context** *PLoS Biol* **4** https://doi.org/10.1371/journal.pbio.0040115

[34] American Diabetes Association (2015) **(2) Classification and diagnosis of diabetes** *Diabetes Care* **38** https://doi.org/10.2337/dc15-S005

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Reviewer #1 (Public review):

This is a well-done clinical study which provides new information on the effects of metabolic disturbances in the human skeleton. 63 postmenopausal women undergoing hip arthroplasty, consisting of T2D with obesity; obesity alone; and neither T2D nor obesity were studied. Most of the findings relate to T2D. Increased serum TNF-α was found in T2D, as well as increased bone gene expression of TNF- α, which was associated with reduced expression of Wnt pathway genes. mRNA levels of certain of the cytokines correlated with Wnt signaling components. In addition, the increased serum TNF- α in T2D was associated with reduced Young's modulus, a measure of bone strength. A strength of this paper is that it provides information in an area that is not well-understood. However, there are a number of concerns that warrant direct addressing.

(1) Can the authors speculate why the changes in cytokines and Wnt expression do not impact bone microarchitecture?

(2) The authors state that they are showing an association between inflammation and bone strength via the regulation of Wnt signaling. However they have only shown here that serum cytokines correlate with bone strength. It is true that the authors have previously shown that Wnt signaling correlated with bone strength. But here it would be useful to show if bone strength is also correlated with inflammatory genes.

(3) AGEs increase inflammation (by binding to RAGE which triggers an inflammatory cascade). AGEs might also increase SOST. From their previous work, it seems that the authors have bone AGE measures on these patients and they have shown their relationship with SOST. Do the increased AGEs relate to inflammation as measured by serum and bone expression?

(4) Were bone turnover markers done to show how the inflammation and Wnt findings relate to bone resorption and formation?

(5) RNA integrity values should be reported to confirm that the RNA has not degraded.

(6) The discussion of adiponectin could be clearer (studies are cited that show both positive and negative effects). Please clarify that adiponectin effects on bone are complex and what they are.

(7) Were patients excluded for prior as well as current antiresorptive medication use?

(8) Fig 4A. correlation between SOST mRNA and TNF-a mRNA seems to be driven by 1 outlier. Does the relationship persist if it is removed?

https://doi.org/10.7554/eLife.102146.1.sa2

Reviewer #2 (Public review):

Summary:

Chronic inflammation of the bone microenvironment conferred by T2DM and obesity may inhibit bone formation and bone strength by decreasing the ratio of Wnt ligands/Wnt inhibitors.

The authors studied 63 postmenopausal women (age >65 years) undergoing hip replacement for osteoarthritis. These were grouped into T2DM and obesity, obesity only, and normal subjects. A set of inflammatory markers was measured in the serum and gene expression of members of the Wnt system in the bone tissue. Bone samples were assessed by micro-CT.

While TNF-α serum levels were higher in T2DM, IL-6 levels were higher in obesity as compared to control. In the bone compartment the most consistent finding was decreased mRNA levels for WNt10b and increased sclerostin mRNA levels, translating into a suppressed Wnt-to-Wnt inhibitor ratio, which was associated with low bone strength.

Strengths:

The study includes clinically well-characterized subjects of three defined subgroups. The analyses were comprehensive.

Weaknesses:

Including data or information on the Wnt inhibitor Dkk1 would be instructive. Analysis were limited to mRNA studies. Validation of protein levels would be supportive (although technically challenging).

https://doi.org/10.7554/eLife.102146.1.sa1

Reviewer #3 (Public review):

In this manuscript, the authors examine circulating and bone parameters in patients with T2DM or obesity vs control subjects. Based on their findings they conclude that increased inflammation in bone of subjects with T2DM and obesity is negatively correlated with Wnt pathway signaling and bone strength.

Overall, this is a well done clinical study that provides further insights into the pathogenesis of bone loss associated with T2DM. However, there are a number of issues that the authors should address:

(1) The major conceptual problem is that the alterations in circulating and bone factors they observed would predominantly affect bone turnover and thus, bone mass. But bone mass is preserved in T2DM (as their own data show). They postulate that their findings lead to impaired bone quality, but it is not clear how this would occur. For example, the impairment in bone quality could be due to the accumulation of AGEs in bone in T2DM, and the correlations observed be true but unrelated. Along these lines, were serum or bone AGEs measured - and if not, is it possible for the authors to do so? At the least, this issue should be fully addressed in the Discussion if the authors are unable to provide additional data to address this.

(2) The T2DM patients were extremely well controlled. This may have limited some of the differences between groups. Was it not possible to select a group of less well-controlled patients - that is more the norm? This may also explain why the biomechanical indices in Table 3 were only marginally different in the T2DM vs the other groups. This point should also be addressed.

(3) The authors found some interesting differences in bone sclerostin levels. Were circulating sclerostin levels measured? This data would be of interest and should be provided.

(4) Fig 4A - the correlation between TNFa and SOST seems to be driven by one highly influential point. What happens if this point is removed? Is this point a formal statistical outlier? Please check this.

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