Body composition is associated with bone and glucose metabolism in postmenopausal women with type 2 diabetes mellitus

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Running tittle: Body composition and bone and glucose metabolism in type 2 diabetes mellitus

Summary

Type 2 diabetes mellitus (T2DM) is associated with increased fracture risk; the underlying mechanism remains unexplained. This study aimed to investigate the relationships between body composition and bone and glucose metabolism in postmenopausal women with T2DM. Dual-energy X-ray absorptiometry was used to measure bone mineral density (BMD) and body composition. A total of 68 postmenopausal women with T2DM and 71 controls were eligible for the study. In contrast to normal BMD in T2DM, a similar prevalence of low-trauma fractures was observed in both groups. T2DM women had significantly higher Trunk fat% and A/G ratio and significantly lower Legs LM% and Legs FM%. Legs LM% was significantly lower in fractured T2DM group and negatively correlated with glycaemia and HbA1c ($p<0.01$). Serum osteocalcin was significantly lower in T2DM and inversely correlated with FM%, Trunk FM% and A/G ratio ($p < 0.01$) and positively correlated with Legs FM% and total LM% ($p <0.05$). In conclusion, abdominal obesity and decrease in muscle mass may contribute to low bone formation in T2DM women. Further research is needed to unravel underlying pathophysiological mechanisms and to determine whether maintenance of muscle mass, especially in the lower extremities and/or reduction of central fat mass can prevent fractures.

Key words: Bone mineral density, Body composition, Fat mass, Lean mass, Type 2 diabetes mellitus

Introduction

Type 2 diabetes mellitus (T2DM) and osteoporosis belongs to the most prevalent metabolic disorders occurring mostly in middle-age and older adults, respectively. Although osteoporosis and T2DM have been viewed as separate diseases, accumulating evidence indicates that similar pathophysiological mechanisms underlie both of them (Starup-Linde and Vestergaard 2015). Nowadays, there is evidence that patients with T2DM have an increased risk of fractures compared with those without diabetes (Yamamoto *et al.* 2009; Martinez-Laguna *et al.* 2015). Despite the increased fracture risk, bone mineral density (BMD) is generally higher in patients with T2DM (Yamamoto *et al.* 2009). The underlying mechanisms of increased fracture risk in T2DM are not fully understood. T2DM is frequently accompanied by changes in body composition, including a decrease in muscle mass and strength, particularly in lower extremities and increase in fat mass (Moseley *et al.* 2011; Park *et al.* 2006). The loss of muscle strength and/or muscle mass might contribute to fractures in T2DM patients. In both cross-sectional and longitudinal studies, accelerated loss of muscle mass and strength is recorded in individuals with diabetes, is greater with longer diabetes duration or higher HbA_{1c}, and is attenuated by use of insulin sensitizers (Park *et al*. 2009; Lee *et al.* 2011; Kalyani *et al.* 2014). The pathogenesis of diabetes-related muscle loss is multifactorial and has been attributed to hyperglycemia, oxidative stress, inflammation, endocrine changes, inactivity, and the accumulation of advanced glycation endproducts (AGEs) (Kalyani *et al.* 2015; Tanaka *et al.* 2015).

Several studies have indicated that also fat mass is related to BMD and fracture risk (Ng *et al.* 2013; Caffarelli *et al.* 2014). Moreover, recent studies in animals suggest that fat-muscle-bone relationships may be related to circulating osteocalcin, an osteocyte and osteoblasts specific peptide (Kanazawa *et al*. 2015). Osteocalcin knockout mice have increased fat mass and decreased insulin sensitivity (Lee *et al.* 2014), whereas treatment of

wild-type mice with undercarboxylated osteocalcin leads to decreased fat mass and improved insulin sensitivity (Ferron *et al.* 2014). Several clinical studies (Kindblom *et al.* 2009; Kanazawa *et al*. 2011) have shown that serum total or under-carboxylated osteocalcin concentrations were associated with glucose metabolism and fat mass in patients with T2DM or in non-diabetes subjects. These experimental and clinical findings suggest that osteocalcin may play an important role both in bone and glucose-fat metabolism.

Measuring body composition by dual energy X-ray absorptiometry (DXA) has the ability to accurately identify fat mass and fat-free mass (lean mass) and their distribution throughout the body with high precision (Lohman *et al.* 2009). In the present study, we therefore examined the relationships of BMD and biochemical variables of bone and glucose metabolism with body composition indices as assessed by DXA in postmenopausal women with and without T2DM.

Methods

Subjects

Postmenopausal women with T2DM on anti-diabetic medication or newly detected T2DM as well as control group of postmenopausal women without T2DM, who attended a preventive bone mineral density (BMD) measurement, were selected prospectively between August 2012 and July 2014. Exclusion criteria were abnormal serum calcium level, serum creatinine level > 110 μmol/l, estimated glomerular filtration rate (eGFR) < 1 ml/s/1.73 m^2 and proteinuria, diseases other than osteoporosis and T2DM that would interfere with bone metabolism such as diabetic nephropathy, primary hyperparathyroidism, liver disease, malabsorption; or use of any other medication affecting bone metabolism within the 5 years prior the selection, such as bisphosphonates, raloxifene, strontium ranelate, hormone replacement therapy, glucocorticoids, thiazolidinedione or active vitamin D supplements. The information on the

risk factors for osteoporosis, namely family history of osteoporosis and a personal history of any kind of bone fracture as an adult (after menopause), physical activity, alcohol intake and smoking were obtained by questionnaires and medical records. Patients with a history of immobility were excluded from the study. A total of 68 postmenopausal women with T2DM (mean age 63 ± 6.5 years) were eligible for the analysis. The majority of T2DM patients (53%) were treated by metformin; 22% of patients were treated by combination of metformin with gliptins or sulfonylurea derivatives and 3% of patients were taking sulfonylurea derivatives. Moreover, 22% of patients had newly detected T2DM without T2DM treatment. The diabetics did not have the chronic complications and no signs of renal complications at the time of examination. There were no significant differences in BMD, body composition or measured biochemical markers between treatment groups or between treated and untreated patients with T2DM. The same exclusion criteria were respected also for the control group. A total of 71 age- and weight-matched postmenopausal control women (mean age 63 ± 8.8 years), were eligible for the study. The study was undertaken with the understanding and written consent of each subject, with the approval of the Ethics Committee of the General University Hospital and First Faculty of Medicine, and within compliance of the National Legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki).

Anthropometric measurement

Standing height and weight measurements were completed with participants wearing lightweight clothing and no shoes. Height was obtained with a stadiometer. Weight was measured on a calibrated digital scale. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

Body composition and bone measures

Body composition, total body bone mineral content (TBBMC) and bone mineral density

(BMD) was measured at the lumbar spine (L1-L4), total femur (TF), femoral neck (FN), distal radius (DR) and whole body (WB) in all participants by using a dual energy X-ray absorptiometry (DXA) densitometer (Discovery A, Hologic, Inc, MA, USA, Software version: Apex 3.5.1). Patients and control group were measured on the same densitometer. The short-term precision in vivo errors for the lumbar spine (L1-L4), total femur, and femoral neck were 0.7 %, 0.9 % and 1.9 %, respectively; the long-term precision in vitro error was 0.32 %. We measured body-composition variables from the whole body scan. The CVs for fat and lean mass by DXA are 1.9 % and 2.6 %, respectively, for our site. Taking into account the large variation of body composition parameters, we expressed the body composition variables also as mass percentages. Total and regional body composition variables were calculated as follows:

Fat mass indices: *Fat mass index (FMI)* = Total body fat mass/ height (kg/m^2); *Percentage of total body fat mass (FM %)* = total body fat mass/ whole body mass x 100; *Percentage of trunk fat (Trunk fat %)* = trunk fat mass/total fat mass x 100; *Legs fat mass index (Legs FMI)* = (right leg + left leg fat mass) /height (kg/m^2); *Percentage of total legs fat mass (Legs FM %)* = (right leg + left leg fat mass)/total fat mass x 100; *Trunk/Legs FM ratio* = trunk fat mass/ legs fat mass; *Android to gynoid ratio (A/G ratio)* was determined by using fat percentage in A and in the G regions. Android FM was defined as adipose deposition around the abdomen; whereas, gynoid FM was adipose tissue accumulating around the hips. The regions of interest (ROI) were defined using the software provided by the Hologic manufacture.

Lean mass indices: *Lean mass index (LMI)* = total body lean mass/ height (kg/m^2); *Percentage of total body lean mass (LM %)* = total body lean mass/ whole body mass x 100; *Legs lean mass index (Legs LMI)* = (right leg + left leg lean mass)/ height (kg/m^2); *Percentage of total leg lean mass (Legs LM %)* = (right leg + left leg lean mass)/ total lean

mass x 100

The data from the third National Health and Nutrition Examination Survey (NHANES III) were used as the reference sample to calculate the T scores, as recommended by Kanis and Gluer (Kanis and Gluer 2000). According to the criterion set by the World Health Organization, a subject was classified as having osteopenia or osteoporosis if BMD was below 1.0 (T score <-1.0) or 2.5 standard deviations (T score ≤ -2.5) below the young reference population, respectively. Gender-specific reference data were used to calculate the T-scores.

Laboratory analyses

Laboratory analysis that allows inclusion and exclusion criteria was performed in all patients and controls, however due to either non-compliance with the preanalytical phase or refusal of repeated blood sampling analysis for bone turnover markers and sRAGE was done only in a subset of 50 women with T2DM and 52 nondiabetic controls. Venous blood samples were taken after an overnight fast. Routine biochemical analyses were performed with fresh samples; other aliquots were stored at -70ºC before being analyzed. The serum glycated hemoglobin (HbA1c) concentrations were assessed by high performance liquid chromatography. The serum fasting glucose was measured by enzymatic colorimetric (GOD-PAP) method. Fasting serum sRAGE levels were measured by sandwich ELISA (enzyme-linked immunosorbent assay) using standard kits Quantikine, RD Systems, Minneapolis, USA, according to the manufacturer's protocol. The serum concentrations of serum intact parathyroid hormone (PTH) and total 25-hydroxyvitamin D (25-OHD) as well as bone turnover markers serum C-terminal telopeptide of collagen I (βCTX), serum intact N-terminal propeptide of type I procollagen (PINP), and serum N-MID Osteocalcin were measured using the electrochemiluminescence-based immunoanalysis (Modular; Roche Diagnostics, Germany). The serum concentrations of tartrate-resistant acid phosphatase isoform 5b (TRAP

5b) was measured by using enzymoimmunoassay (BoneTRAP Assay, Immunodiagnostic Systems Limited, Boldon, UK).

Statistical analysis

All analyses were conducted using JMP®10.0.0 statistical software (Copyright © 2012 SAS Institute Inc.). Data were expressed by means and standard deviations if not otherwise stated. Differences between groups were calculated by Student's t-test. Correlation analyses were performed using Spearman's ranked correlation coefficient. To assess whether relationship with body composition indices or measured biochemical variables is specific for theses parameters, adjustment for age and diabetes duration in subsequent analysis by multiple linear regression was done. All statistical tests were two-tailed and $p<0.05$ was considered statistically significant.

Results

Study population

Table 1 shows the anthropometric characteristics and measured biochemical parameters of the postmenopausal women with and without T2DM. Women with T2DM did not differ from control subjects in age, weight or height; however body mass index (BMI) was slightly higher in T2DM women ($p = 0.0497$). The prevalence of obesity (defined by BMI >30 kg/m^2) was 59% in T2DM postmenopausal women and 48% in control group. *Fat mass and lean mass indices*

The detailed differences in body composition indices of the two groups are presented in Table 2. We have found no significant difference in total fat mass indices FMI (kg/m^2) and FM% between T2DM and control subjects. However, a significantly different distribution of fat mass indices between T2DM women and control subjects was found. Diabetic women had

significantly higher Trunk fat % (52.3 ± 5.3 % vs. 46.3 ± 6.1 %, p <0.001) and A/G ratio (1.05 ± 0.13 vs. 0.94 ± 0.16; p <0.001) as well as Trunk/legs FM ratio (1.75 ± 0.50 vs. 1.31 ± 0.38; p <0.001) versus control subjects. On the other hand, Legs FM% was significantly lower in T2DM women (31.34 ± 5.3 vs. 37.04 ± 5.89, p < 0.001). When analyzing the lean mass indices, we have found no significant difference in total LM% between T2DM patients versus control subjects; however, women with T2DM had slightly higher LMI versus control subjects (p<0.05). Legs LMI was not significantly different between patients and control subjects; however, the Legs LM% was significantly lower in T2DM patients versus controls (31.32 ± 2.0 % vs. 32.34 ± 1.7%; p < 0.01).

Bone mineral density, fractures and biochemical markers of bone turnover

In our cohort of 68 postmenopausal women with T2DM, 32.4 % of women had normal BMD, 48.5 % osteopenia and 19.1 % osteoporosis, in control nondiabetic women 28.2 %, 50.7 % and 21.1% of subjects, resp. (Tab. 2). The prevalence of low-trauma vertebral and non-vertebral fractures in T2DM women was 11.8% and 23.5%, resp. and 12.7% and 19.7%, resp. in controls. We found no significant differences in measured BMD regions between T2DM women and nondiabetic controls (Tab. 2) and between fractured and non-fractured groups (Tab. 3). Circulating biochemical markers of bone formation, PINP and osteocalcin was lower in T2DM women, although only serum osteocalcin level reached a significant difference between T2DM and controls (the mean 17.9 ± 6.7 ug/l and 21.6 ± 6.8 ug/l, resp., p = 0.015) (Tab. 1). The concentrations of bone resorption markers serum βCTX and TRAP5b as well as serum sRAGE were not significantly different between groups. T2DM postmenopausal women had significantly lower 25OHD levels when compared to controls (the mean 52.0 ± 25.0 vs. 72.5 ± 29.5 nmol/l; p <0.001) (Tab.1).

No noninvasive method for evaluating bone quality is clinically available at present;

however, the presence of low trauma fractures could be used for the assessment of bone quality in individual patients. Next, we therefore compared body composition indices and biochemical variables between women with and without low trauma fractures (Tab. 3). Body height, body weight and BMI were not significantly different between women with and without fractures in both groups. As shown in Table 3, BMD at any site were also similar between women with and without fractures. As for body composition, legs LM% was significantly lower in the T2DM group with fractures compared with those without it. Legs LMI, LM% and fat mass indices were not significantly different between groups with and without fractures (Tab. 3). Bone markers were not significantly different between groups with and without fractures, although serum osteocalcin and serum TRAP 5b tend to be lower in T2DM fracture group (15.8 ± 5.8 ug/l vs. 18.5 ± 6.8 and 2.4 ± 1.2 vs. 3.0 ± 0.09, osteocalcin and TRAP5b, resp.) (Tab. 3). Only 25OHD level was significantly higher in the T2DM group with fractures (p=0.008) (Tab. 3).

Correlation analysis

Firstly, we investigated if indices of fat and muscle mass correlated with BMD. TF BMD was positively correlated with lean mass indices as well as with some fat mass indices both in T2DM and controls. The Spearman's ranked correlation coefficient in T2DM women were as follows: LMI (0.416, p = 0.0005), LM% (0.327, p= 0.0267), Legs LMI (0.447, p = 0.0002) and Legs LM% (0.287, p = 0.0217) and FMI (0.310, p = 0.0113) and Legs FMI (0.271, p = 0.0280). To assess whether relationship with body composition indices or measured biochemical variables is specific for these parameters, adjustments for age and diabetes duration was done in subsequent analysis by multiple linear regression. The correlation between TF BMD and LMI, FMI and Legs LMI (Fig. 1) remained significant in T2DM (r^2=0.318, p = 0.0408; r^2=0.313, p = 0.0125 and r^2= 0.330, p = 0.0054, resp.) as well as in controls (r^2= 0.206, p = 0.004; r^2= 0.181, p = 0.0124 and r^2= 0.198, p = 0.0056, resp.),

whereas the relationship between Legs FMI and TF BMD was no longer significant in T2DM group (Fig. 1). Although, both LM and FM indices were significantly related to TF BMD, the total LMI and Legs LMI seems to be more closely related to TF BMD ($p < 0.001$).

Secondly, we investigated if indices of fat and muscle mass correlated with measured biochemical parameters including HbA_{1c}, glucose, and bone turnover markers, namely osteocalcin. Total LM%, FM% as well as Legs FMI negatively correlated with serum fasting glucose in control group after adjustment for age (r^2=0.386, 0.422 and 0.334, resp.). In T2DM women only Legs LM% negatively correlated with serum fasting glucose ($r^2 = 0.244$, $p = 0.004$) and with HbA1c ($r^2 = 0.244$, $p = 0.009$) even after adjustments for age and diabetes duration. Serum osteocalcin in T2DM women negatively correlated with fat mass indices even after adjustments for age and diabetes duration: FMI (r^2 =0.237, p= 0.0212), FM% (r^2 =0.255, p =0.0120; Fig. 3), A/G ratio (r^2 =0.283, p= 0.0053), Trunk fat % (r^2 =0.285, p = 0.0045; Fig. 2), Trunk/fat legs fat mass (r^2 =0.243, p= 0.0178) and positively correlated with Legs FM% (r^2 =0.120; p=0.017) (Fig. 2) and total LM % (r^2 =0.248, p= 0.0152) (Fig. 3). In the control group, our results showed similar associations (data not shown). There were no significant associations of 25OHD concentrations with respect to serum calcium, intact PTH, creatinine or eGFR; however 25OHD was negatively associated with total FM in T2DM postmenopausal women ($r = 0.302$; $p = 0.031$) as well as in controls ($r = 0.256$, $p = 0.044$).

Discussion

Numbers of patients with osteoporosis or T2DM increase with age and both disorders adversely affect the health by causing fractures and vascular complications, respectively. Although osteoporosis and T2DM have been viewed as separate diseases, accumulating evidence indicates that similar pathophysiological mechanisms underlie both of them. The present study explored the impact of fat and muscle mass indices (measured by DXA) on bone and glucose metabolism in postmenopausal women with and without T2DM.

Our results clearly showed significant differences in body composition between postmenopausal women with and without T2DM with its unfavorable distribution in T2DM women characterized by more central fat and decreased percentages of legs fat mass and legs lean mass. These results are in accordance with previous studies that have shown that patients with T2DM have central obesity as well as reduction of appendicular lean mass, particularly in the lower extremities, compared with subjects without it (Leenders *et al.* 2013; Moseley *et al.* 2011). We demonstrated that Legs LMI as well as total LMI or FMI significantly correlated with TF BMD both in T2DM women and controls even after adjusting for age and diabetes duration. As for body composition between fractured and non- fracture groups, only legs LM% was significantly lower in the T2DM group with fractures compared with those without it (Tab. 3). These data suggest that lower muscle mass, especially in the lower limbs may be a risk factor for loss of hip BMD in postmenopausal women with T2DM, considering its future impacts on quality of life, physical disability and fracture risk in these patients.

There were no significant differences in total body BMC or BMD between the T2DM and the controls or between fractured and non-fracture groups. These results are in accordance with previous studies which demonstrated that BMD is generally higher in those with T2DM compared to those without (Vestergaard 2007). In contrast to normal or higher BMD in those with T2DM we documented a similar prevalence of low trauma fractures between postmenopausal women with and without T2DM (Tab. 2). However, it is well known that DXA technique has its own limitations. Further techniques, such as peripheral quantitative computed tomography which allows for separate assessment of the trabecular and cortical compartments of the bone, may provide better insight into the trabecular-cortical bone relationships (Sukumar *et al.* 2012); e.g. in a recent study, T2DM was associated with unfavorable cortical bone microarchitecture at the distal radius (Yu *et al.* 2015).

Previous studies have shown that low vitamin D, physical inactivity, hyperglycemia or

AGEs are associated with muscle mass or muscle strength reduction (Tanaka *et al.* 2015). In the present study, the multivariate analysis confirms the negative correlation between Legs LM% and serum HbA1c or fasting glucose in T2DM group. These results indicate that hyperglycemia might be a risk factor for loss of muscle mass in postmenopausal women with T2DM (Kalyani *et al.* 2014). More recently, Kalyani *et al*. reported that hyperglycemia measured by HbA_{1c} is associated with the lower muscle strength (Kalyani *et al.*2015). Reduced insulin signaling in T2DM leads to decreased protein synthesis and increased protein degradation, which can lead to reduced muscle mass. In addition, chronic inflammation, oxidative damage, and mitochondria dysfunction have also been suggested to be associated with both diabetes mellitus and reduced muscle quality (muscle strength/muscle mass). Among other risk factors, vitamin D deficiency may influence muscle quality.

In accordance with previous studies (Miñambres *et al.* 2015) we found a significantly higher prevalence of hypovitaminosis D (25OHD levels < 75 nmol/l) in postmenopausal women with T2DM than in non-diabetic controls (82.4% vs. 54.2%) (Tab.1). Several studies have shown that obesity is associated with low serum 25OHD levels (Shantavasinkul *et al.* 2015). In our study, we found a significant negative association between 25OH D levels and total body fat mass. This inverse 25OHD and fat mass relationship has been explained by "trapping" of the vitamin D parent compound, cholecalciferol, in adipose tissue. In the present study, the serum 25OHD levels are increased in T2DM patients with fracture compared to T2DM patients without fracture but they do not significantly differ from controls. We have no clear explanation for this result; however, direct measurement of sun exposure, seasonal variability as well as indoor or outdoor exercise patterns was not analyzed. Only three patients with fracture and five patients without fracture were using a low daily dose of cholecalciferol (500-1000 IU).

In the present study, the positive correlation between BMD and total fat mass indices

(FMI, FM %) both in T2DM women and controls indicates that total fat mass is associated with BMD and may therefore protect against osteoporosis (De Laet *et al.* 2005); however, newer studies have demonstrated that abdominal obesity assessed by quantitative computed tomography, which can differentiate between visceral and subcutaneous adipose tissue, is inversely related to BMD (Bredella *et al.* 2011; Sheu *et al*. 2011). In our study, we demonstrated significantly higher trunk fat mass indices in T2DM women as compared with nondiabetic women (Tab. 1). There was no significant association between measured trunk fat indices and BMD and no significant differences in trunk fat mass indices between groups with and without fractures. No association between trunk fat mass and BMD in our study may be because most of women were overweight or obese and a higher fat mass distribution in soft tissue around the bone and within the marrow may affect DXA measurements (Hangartner *et al.* 1990). Previous DXA study in adolescent athletes found an inverse association between trunk fat mass and BMD (Ackerman *et al*. 2011). More importantly, recent bone biopsy study by Cohen *et al.* demonstrated that higher trunk fat mass is associated with inferior bone quality, namely with markedly lower static and dynamic parameters of bone formation and with thinner trabeculae and higher cortical porosity (Cohen *et al.* 2013). These results suggest that abdominal obesity may have an important influence on bone remodeling and bone quality, which may occur independently on BMD.

We found that serum bone formation marker osteocalcin was significantly lower in T2DM postmenopausal women (Tab. 1). The marker of bone collagen synthesis serum PINP and bone resorption markers, serum βCTX and TRAP5b, were also lower (although differences did not reach statistical significance), suggesting a decrease in bone remodeling. Several other studies have evaluated the relationships between obesity and serum bone formation markers in premenopausal and postmenopausal women and found that bone turnover markers, such as osteocalcin were lower in obese than normal-weight subjects

(Bredella *et al.* 2011; Lee *et al.* 2012, Garcia-Martin A *et al*. 2011). Moreover, in our study serum osteocalcin was inversely associated with total FM% and trunk fat mass indices (Trunk FM%, A/G ratio and Trunk/legs FM ratio) in postmenopausal women with and without T2DM even after adjustments for age and diabetes duration (Fig. 2, 3). In contrast, accumulation of fat in the legs (Legs FM %) positively associated with osteocalcin levels (Fig. 2). In addition, there was a significant positive correlation between serum osteocalcin and total LM % (Fig. 3). Although, the mechanism(s) by which fat mass, particularly central fat mass affects the bone remodeling is unknown (Cohen *et al*. 2013), studies in animal models strongly support the view that osteocalcin may play an important role in fat-bone relationships (Lee *et al.* 2014). On the other hand, adipose tissue is also considered as endocrine organ, which may produce osteocalcin (Foresta *et al.* 2010). There was a strong positive correlation between osteocalcin and PINP or measured bone resorption markers (data not shown), which suggests coupling between bone formation and bone resorption; however, our results found that only circulating osteocalcin, but not PINP, βCTX or TRAP5b, were associated with fat mass indices. These findings suggest that mature osteoblast or osteocytes function is specifically affected by glucose metabolism in postmenopausal women.

The concentrations of serum sRAGE were not significantly different between T2DM and control group or between patients with and without fracture. At present, it is unclear whether circulating sRAGE is elevated in all patients with T2DM – as higher, lower, and even similar levels of sRAGE have been reported in patients with T2DM compared with control nondiabetic subjects (Yamagishi and Matsui, 2010). These contradictory findings may be at least partly explained by differences in the renal function in diabetic patients in these studies. In our study, there were no signs of renal complications in T2DM patients or controls at the time of examination, which could at least partly explain no differences in sRAGE between groups.

Limitations

The present study was observational, and provides limited insight to unravel underlying pathophysiological mechanisms of the observed relationships. We studied only subjects who visited our hospital, a tertiary center, for evaluation and treatment diabetes mellitus and osteoporosis. Therefore, the subjects enrolled in the present study might not be representative of Czech postmenopausal women with the disorders. Measuring body composition by dual energy X-ray absorptiometry (DXA) is a noninvasive and valid method that allows separation of the body mass into bone mass, fat mass, and lean (fat free) mass; however, this technique has its own limitations. Although, trunk fat measured by DXA is considered a surrogate for visceral fat mass, it cannot differentiate between visceral and subcutaneous adipose tissue and includes fat in the rib and pelvis compartments. In fact, the variable fat mass distribution in soft tissues in obese women may have interfered with our ability to detect differences with the 2-dimensional DXA assessment.

Conclusion

In contrast to normal or higher BMD in those with T2DM, we documented a similar prevalence of low trauma fractures between postmenopausal women with and without T2DM. These findings suggest that poor bone quality rather than decreased bone mass may define bone fragility that causes low trauma fractures in T2DM. In postmenopausal women with T2DM, lower muscle mass (total LM%) and higher trunk fat mass was accompanied by significantly lower serum osteocalcin levels, a specific marker of osteoblast and osteocytes activity. These results suggest that abdominal obesity and lower muscle mass might be related to low bone formation in T2DM women. Moreover, in our study, legs muscle mass (Legs LM%) was significantly lower in fractured T2DM women as compared with non-fractured patients. The hyperglycemia contributes to decline in muscle mass, especially in the lower

extremities, which may further contribute to poor bone quality in T2DM women. Further research is needed to unravel underlying pathophysiological mechanisms and to determine whether maintenance of muscle mass and/or reduction of central fat mass can prevent fractures and improve the glucose metabolism in T2DM postmenopausal women.

Conflict of Interest

There is no conflict of interest.

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Tab. 1. The anthropometric characteristics and measured biochemical variables of the postmenopausal women with and without T2DM

	Postmenopausal Women with T2DM	**Nondiabetic controls**	**P value**
N	68	71	
Age (yrs)	62.96 ± 6.5	63.23 ± 8.8	ns
YSM (yrs)	13.36 ± 7.5	13.58 ± 9.2	ns
Weight (kg)	86.04 ± 20.7	79.35 ± 21.9	ns
Height (m)	1.61 ± 0.06	1.61 ± 0.07	ns
BMI (kg/m^2)	33.21 ± 8.2	30.51 ± 7.8	0.049
S-HbA_{1c} (mmol/mol)	52.57 ± 14.3	37.87 ± 4	< 0.001
S-glucose (mmol/l)	7.1 ± 2.0	5.2 ± 0.5	< 0.001
Diabetes duration (yrs)	7.36 ± 6.9	-	-
S-Ca (mmol/l)	2.30 ± 0.1	2.27 ± 0.1	ns
S-Creatinine (μmol/l)	67.5 ± 11.8	70.0 ±10.6	ns
eGFR ($ml/s/1.73m^2$)	1.24 ± 0.18	1.19 ± 0.16	ns
S-25OHD (nmol/l)	52.0 ± 25.0	72.5 ± 29.5	<0.001
S-intact PTH (pmol/l)	5.2 ± 2.5	4.9 ± 1.9	ns
Bone markers			
N	50	52	
S-TRAP 5b (IU/l)	2.7 ± 1.1	2.6 ± 1.1	ns
S-βCTX (ng/l)	377.0 ± 188.7	408.0 ± 158.1	ns
S-PINP (μg/l)	39.4 ± 16.7	45.5 ± 17.3	0.069
S-Osteocalcin (μg/l)	17.9 ± 6.7	21.6 ± 6.8	0.015
S-sRAGE (ng/l)	1371.0 ± 597.1	1504.6 ± 597.3	ns

Data are presented as mean values (±SD).

Abbreviations: BMI: Body Mass Index, eGFR: estimated glomerular filtration rate; 25 OHD: 25-hydroxyvitamin D; intact PTH: intact parathyroid hormone; TRAP5b: tartrate-resistant acid phosphatase isoform 5b; βCTX: C-terminal telopeptide of collagen type I; PINP: intact N-terminal propeptide of type I procollagen, sRAGE: soluble receptor for AGEs

Tab. 2. Bone mineral density (BMD), percentages of low BMD and number of low-trauma fractures, and fat and lean mass distribution in postmenopausal women with and without T2DM

	Postmenopausal women with T2DM	**Nondiabetic controls**	**P value**
N	68	71	
Age (yrs)	62.96 ± 6.5	63.23 ± 8.8	ns
YSM (yrs)	13.36 ± 7.5	13.58 ± 9.2	ns
Weight (kg)	86.04 ± 20.7	79.35 ± 21.9	ns
Height (m)	1.61 ± 0.06	1.61 ± 0.07	ns
BMI (kg/m^2)	33.21 ± 8.2	30.51 ± 7.8	0.049
BMD Lumbar Spine (g/cm^2)	0.96 ± 0.16	0.93 ± 0.14	ns
BMD Femoral neck (g/cm^2)	0.74 ± 0.12	0.74 ± 0.11	ns
BMD Total Femur (g/cm^2)	0.94 ± 0.14	0.91 ± 0.15	ns
BMD Distal Radius (g/cm^2)	0.63 ± 0.07	0.62 ± 0.07	ns
BMD Whole Body (g/cm^2)	1.07 ± 0.12	1.02 ± 0.19	ns
Total Body Bone Mineral Content (g)	2115 ± 348	2087 ± 326	ns
Normal (T-score ≥ -1.0)	32.4%	28.2%	ns
Osteopenia (T score < -1.0 and > -2.5)	48.5%	50.7%	ns
Osteoporosis (T-score ≤ -2.5)	19.1%	21.1%	ns
Vertebral fractures (No. of patients)	8 (7)	9 (6)	-
Non vertebral fractures (No. of patients)	16 (13)	14 (11)	-
- humerus	-	1 (1)	-
- forearm	8 (8)	10 (9)	-
- ankle/tibia	8 (5)	3 (2)	-
Fat mass indices			
FMI (kg/m^2)	14.4 ± 5.5	13.35 ± 5.2	ns
FM %	41.12 ± 4.9	41.19 ± 5.97	ns
A/G ratio	1.05 ± 0.13	0.94 ± 0.16	< 0.001
Trunk fat%	52.3 ± 5.27	46.32 ± 6.07	< 0.001
Trunk/legs FM ratio	1.75 ±0.50	1.31 ± 0.38	< 0.001
Legs FMI (kg/m^2)	4.81 ± 1.67	4.46 ± 1.70	ns
Legs FM %	31.34 ± 5.3	37.04 ± 5.89	< 0.001
Lean mass indices			
LMI (kg/m^2)	18.52 ± 3.9	17.18 ± 2.9	< 0.05
LM %	57.22 ± 4.7	57.94 ± 5.3	ns
Legs LMI(kg/m^2)	6.07 ± 1.5	5.82 ± 1.1	ns
Legs LM %	31.32 ± 2.0	32.34 ± 1.7	< 0.01

Data are presented as mean values (±SD).

Abbreviations: FM: Fat mass; LM: Lean mass; FMI = Fat mass index; FM %: percentage of total body Fat mass; Trunk fat %: percentage of Trunk fat; Legs FM %: percentage of Legs Fat mass; Trunk/Legs FM ratio: Trunk / Legs Fat mass ratio; A/G ratio: Android to gynoid FM ratio; LMI: Lean mass index; LM %: percentage of total body Lean mass; Legs LM %: percentage of Legs lean mass

Tab. 3. Comparison of anthropometric, bone mineral density, body composition and biochemical indices between postmenopausal women with T2DM and controls with (+) and without (-) low-trauma fractures.

	T2DM		**Control**	
	Fracture (+)	**Fracture (-)**	**Fracture (+)**	**Fracture (-)**
N	20	48	16	55
Age (yrs)	65.1 ± 6.2	62.1 ± 6.5	65.0 ± 7.7	62.7 ± 9.1
YSM (yrs)	13.9 ± 6.9	13.2 ± 7.8	17.3 ± 8.4	14.8 ± 19.5
BMI (kg/m^2)	32.7 ± 6.1	33.4 ± 9.1	29.8 ± 5.7	30.7 ± 8.3
Fat mass indices				
FMI (kg/m^2)	14.68 ± 4.13	14.59 ±5.7	12.98 ± 3.8	13.46 ± 5.7
FM %	42.18 ± 5.02	40.65± 4.87	41.13 ± 6.35	41.39 ± 4.6
Trunk fat %	53.47 ± 5.43	51.79 ± 5.18	46.93 ± 4.7	46.15 ± 6.4
Trunk/legs FM ratio	1.85 ± 0.56	1.70 ± 0.46	1.30 ± 0.30	1.31± 0.40
A/G ratio	1.08 ± 0.12	1.03 ± 0.13	0.96 ± 0.11	0.94 ± 0.17
Legs FMI	4.49 ± 1.6	4.56 ± 1.7	4.76 ± 1.2	4.83 ± 1.8
Legs FM%	30.19 ± 5.17	31.84 ± 5.27	37.3 ± 4.9	36.9 ±.6.2
Lean mass indices				
LMI (kg/m^2)	18.4 ± 2.6	18.97 ± 3.5	16.83 ± 2.7	17.28 ± 3.0
LM %	56.59± 4.68	57.48 ± 4.75	58.09 ± 5.8	57.4 ± 3.7
Legs LMI (kg/m^2)	5.89 ± 1.0	6.31 ± 1,36	5.59 ± 0.9	5.90 ± 1.1
Legs LM %	30.58 ± 1.98*	31.84 ± 5.27	32.52 ± 1.6	31.69 ± 2.1
Bone Mineral Density				
Lumbar spine (g/cm^2)	0.957 ± 0.173	0.964 ± 0.156	0.911 ± 0.124	0.947± 0.149
Femoral neck (g/cm^2)	0.709 ± 0.111	0.754 ± 0.126	0.732 ± 0.111	0.746 ± 0.121
Total femur (g/cm^2)	0.901± 0.142	0.961 ± 0.140	0.875 ± 0.112	0.922 ± 0.158
Distal radius (g/cm^2)	0.619 ± 0.082	0.639 ± 0.073	0.615 ± 0.077	0.628 ± 0.072
Whole body (g/cm^2)	1.061± 0.140	1.076 ± 0.118	1.018 ± 0.155	1.043 ± 0.082
S-HbA_{1c} (mmol/mol)	53.1 ± 14.2	51.4 ± 13.8	37.8 ± 4.2	37.9 ± 4.0
S-glucose (mmol/l)	7.3 ± 1.4	7.0 ± 2.2	5.1 ± 0.3	5.2 ± 0.6
Diabetes duration (yrs)	7.6 ± 6.7	7.3 ± 7.0	-	-
eGFR (ml/s/1.73m^2)	1.24 ± 0.17	1.24 ± 0.18	1.19 ± 0.09	1.21 ± 0.17
S-intact PTH (pmol/l)	4.5 ±2.2	5.7 ±2.6	5.3 ±2.1	4.7 ±1.8
S-25OHD (nmol/l)	63.75 ± 21*	45.0 ± 25	77.5 ± 32.75	70.25 ± 28
Bone markers				
N	20	30	16	36
S-TRAP5b (IU/l)	2.4 ± 1.2	3.0 ± 0.9	2.7 ± 1.1	2.6 ± 1.1
S-βCTX (ng/l)	339.7 ± 182.1	391.7 ± 195.2	440.4 ± 134.4	391.8 ± 168.5
S-PINP (μg/l)	37.5 ± 15.4	40.0 ± 17.6	50.9 ± 18.0	42.7 ± 16.5
S-Osteocalcin (μg/l)	15.8 ± 5.8	18.5 ± 6.7	22.7 ± 5.7	21.0 ± 7.3
S-sRAGE (ng/l)	1313.1 ± 511.9	1402.4 ± 643.4	1525.1 ± 463.2	1496.4 ± 650.2

t-test *p <0.05; Data are presented as mean values (±SD).

Abbreviations: BMI: Body Mass Index, eGFR: estimated glomerular filtration rate; 25OHD: 25-hydroxyvitamin D; PTH: intact parathyroid hormone; TRAP5b: tartrate-resistant acid phosphatase isoform 5b; βCTX: C-terminal telopeptide of collagen I; PINP: intact N-terminal propeptide of type I procollagen, sRAGE: soluble receptor for AGEs; FM: Fat mass; FMI: Fat mass index; A/G ratio: Android to Gynoid FM ratio; LM: Lean mass; LMI = Lean mass index

Fig. 1. Relationship between total femur bone mineral density (TF BMD; g/cm^2) and Legs Lean mass index (kg/m^2; upper) or Legs Fat mass index (kg/m^2; bottom) in postmenopausal women with T2DM. Dotted lines: prediction intervals. Multiple linear regression after adjustment for age and diabetes duration.

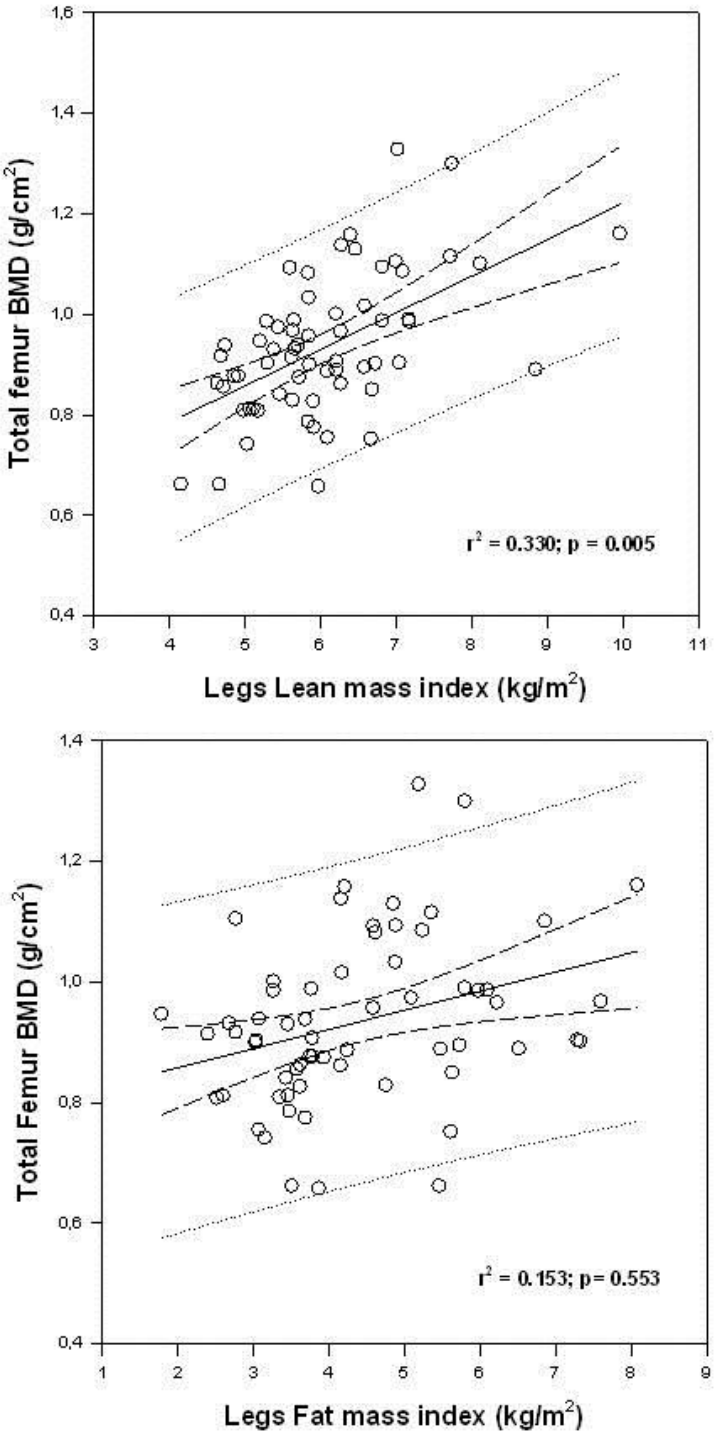

Fig. 2. Relationship between serum osteocalcin and Trunk Fat mass (%; upper) or Legs Fat mass (%; bottom) in postmenopausal women with T2DM. Dotted lines: prediction intervals. Multiple linear regression after adjustment for age and diabetes duration.

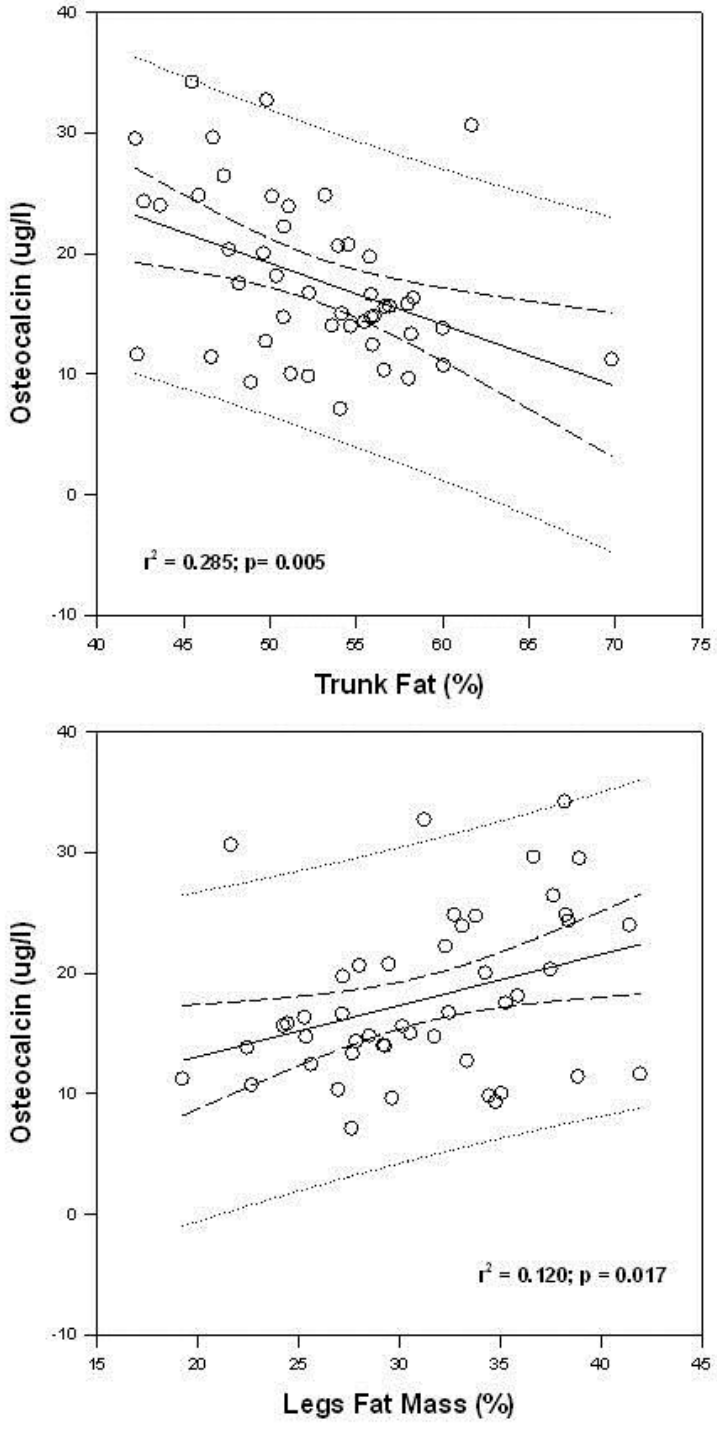

Fig. 3. Relationship between serum osteocalcin and Total Fat mass (%; upper) or Total Lean mass (%; bottom) in postmenopausal women with T2DM. Dotted lines: prediction intervals. Multiple linear regression after adjustment for age and diabetes duration.