Bone 95 (2017) 5–10

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

# Association of osteoglycin and FAM5C with bone turnover markers, bone mineral density, and vertebral fractures in postmenopausal women with type 2 diabetes mellitus

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ARTICLE INFO

*Article history:*
Received 11 August 2016
Revised 18 October 2016
Accepted 6 November 2016
Available online 08 November 2016

*Keywords:*
Osteoglycin
FAM5C
Vertebral fracture
Bone
Diabetes mellitus

ABSTRACT

*Objective:* Accumulating evidence suggests a reciprocal relationship between muscle and bone. Previous in vitro studies showed that the muscle-derived factors, osteoglycin (OGN) and family with sequence similarity 5, member C (FAM5C), regulate osteoblastic differentiation. However, there are no reports investigating the association between circulating OGN and FAM5C and bone metabolism in humans.
*Design:* We conducted a cross-sectional study and investigated the association of serum OGN and FAM5C levels and muscle mass examined by whole-body dual-energy x-ray absorptiometry with bone mineral density (BMD), bone turnover markers, and the presence of vertebral fractures (VFs) in 156 postmenopausal women with type 2 diabetes mellitus (T2DM).
*Results:* Multiple regression analysis adjusted for age, duration of T2DM, body mass index, serum creatinine, and log(hemoglobin A1c) showed that log(OGN) was negatively associated with BMD at the femoral neck ($\beta = -0.17$, $p = 0.014$). Serum OGN levels were higher in subjects with VFs than in those without VFs [mean ± standard deviation (SD): 100.2 ± 84.7 vs. 74.4 ± 31.7 pg/mL, $p = 0.013$]. Moreover, logistic regression analysis adjusted for the confounding factors described above showed that the serum OGN level was positively associated with the presence of VFs (odds ratio = 1.84, 95% confidence interval = 1.03–3.29 per SD increase, $p = 0.039$). In contrast, neither the serum FAM5C level nor muscle mass indices were associated with bone turnover markers and the presence of VFs.
*Conclusions:* The present study showed for the first time that higher serum OGN levels were associated with decreased BMD and increased risk of vertebral fractures in postmenopausal women with T2DM.


## 1. Introduction

Sarcopenia and osteoporosis are aging-related diseases associated with the deterioration of muscle and bone strength, resulting in frailty in elderly people. Therefore, both diseases have become worldwide social issues along with an aging population. Although sarcopenia and osteoporosis are traditionally viewed as separate entities that increase in prevalence with aging, accumulating evidence indicates that some pathological conditions such as accumulated advanced glycation end products (AGEs) [1–5] and decreased insulin-like growth factor-I [6,7] are involved in both sarcopenia and osteoporosis. Furthermore, recent studies have shown an association between muscle and bone tissues that has been recognized as the muscle-bone interaction [8,9]. Previous studies have shown that muscle tissue expresses and secretes various hormones called myokines [8,9]. However, it is still unknown whether circulating myokines are associated with bone metabolism and the risk of fracture in humans.

We have previously shown that osteoglycin (OGN) and family with sequence similarity 5, member C (FAM5C) are important factors linking muscle to bone with comparative DNA microarray analysis using a heterozygous constitutively activating mutation (c.617G>A; p.R206H) in a bone morphogenetic protein (BMP) type I receptor, the activin receptor type I (ACVR1; MIM#102576)/activin-like kinase 2 (ALK2), found in fibrodysplasia ossificans progressiva [10,11]. OGN, a small leucine-rich proteoglycan, was initially isolated from bovine bone as an inducer of matrix mineralization [12]. OGN is expressed and secreted in myoblasts, and it is detectable in human sera [10]. Previously, our in vitro study

*Abbreviations:* OGN, osteoglycin; FAM5C, family with sequence similarity 5, member C; T2DM, type 2 diabetes mellitus; BMD, bone mineral density; LS, lumbar spine; FN, femoral neck; NTx, type I collagen cross-linked N-telopeptide; BMI, body mass index; HbA1c, hemoglobin A1c; LBM, lean body mass; RSMI, relative skeletal muscle mass index; OR, odds ratio.

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http://dx.doi.org/10.1016/j.bone.2016.11.007
8756-3282/

demonstrated that recombinant OGN treatment significantly suppressed the expression of Runx2 and osterix in osteoblastic MC3T3-E1 cells, whereas it enhanced the expression of type 1 collagen (T1C), alkaline phosphatase (ALP), and osteocalcin. Moreover, treatment with conditioned medium collected from OGN-overexpressing myoblasts also suppressed Runx2 and osterix expression and stimulated expression of T1C, ALP, and osteocalcin as well as enhanced the mineralization of MC3T3-E1 cells [10]. On the other hand, FAM5C was identified in the mouse brain as a protein induced by bone morphogenetic protein and retinoic acid signaling [13]. In our previous study, the conditioned medium collected from FAM5C-overexpressing myoblasts increased the expression of osterix, ALP, and osteocalcin in MC3T3-E1 cells, whereas conditioned medium from FAM5C-suppressed myoblasts decreased their expression [11]. These findings suggest that OGN and FAM5C may be important myokines regulating bone metabolism.

Type 2 diabetes mellitus (T2DM) is also a major disease among elderly people, which causes a variety of vascular complications such as diabetic neuropathy, retinopathy, and nephropathy. Osteoporosis has been recently recognized as a complication of diabetes. Previous studies have shown that patients with T2DM have an increased risk of osteoporotic fractures despite normal or higher bone mineral density (BMD) [14,15]. Osteoporotic fractures, especially hip and vertebral fractures, are associated with deteriorated quality of life and mortality [16–18]. Thus, it is important to clarify the risk factors of fractures in T2DM. On the other hand, it is known that sarcopenia is closely linked with osteoporotic fractures because sarcopenia increases the risk of falling [19]. Although it is reported that the prevalence of sarcopenia is higher in patients with T2DM than in those without it [20], it is unclear whether or not reduced muscle mass is associated with the risk of fractures in T2DM.

In the present study, we thus aimed to examine the association of serum levels of OGN and FAM5C and indices of muscle mass with BMD, bone turnover markers, and the presence of vertebral fractures in postmenopausal women with T2DM. Furthermore, we investigated the association of serum levels of OGN and FAM5C with parameters of glucose metabolism in the population.

## 2. Methods

### 2.1. Subjects

The subjects in this cross-sectional study were 156 Japanese postmenopausal women with T2DM (age, 67.5 ± 9.8 years). We consecutively enrolled subjects who visited Shimane University Hospital for evaluation or treatment of T2DM from 2007 to 2013. All women had been without spontaneous menses for >1 year. None of them had renal dysfunction [estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m$^2$] or hyperthyroidism or had taken drugs known to influence bone such as vitamin D, bisphosphonate, thiazolidinedione, and glucocorticoid. Patient characteristics of demographic and biochemical parameters and BMD are shown in Table 1. Among the subjects, 45, 46, 25, 22, and 23 subjects had been taking insulin, sulfonylurea, metformin, dipeptidyl peptidase-4 inhibitors, and α-glucosidase inhibitors, respectively. This study was approved by the ethical review board of Shimane University Faculty of Medicine and complied with the Helsinki Declaration. All subjects agreed to participate in the study and provided written informed consent.

### 2.2. Biochemical measurements

Blood and urine samples were collected after subjects fasted overnight. Biochemical markers were measured by standard methods as previously described [21,22]. Hemoglobin A1c (HbA1c) was determined with high-performance liquid chromatography. The value for HbA1c was estimated as the National Glycohemoglobin Standardization Program equivalent value calculated using the formula HbA1c (%) = HbA1c (Japan Diabetes Society) (%) + 0.4% [23]. Bone-specific alkaline phosphatase (BAP) and urine type I collagen cross-linked N-telopeptide (NTx) levels were measured by enzyme immunoassay and enzyme-linked immunosorbent assay (ELISA), respectively. Total osteocalcin was measured by radioimmunoassay.

**Table 1**
Baseline characteristics of subjects.

| | |
|---|---|
| Number of subjects | 156 |
| Age (years) | 67.5 ± 9.8 |
| BMI (kg/m$^2$) | 24.1 ± 4.6 |
| Serum creatinine (mg/dL) | 0.67 ± 0.26 |
| FPG (mg/dL) | 158.0 ± 62.5 |
| HbA1c (NGSP) (%) | 8.5 ± 2.3 |
| Duration of T2DM (years) | 12.7 ± 10.4 |
| LS BMD (g/cm$^2$) | 0.856 ± 0.189 |
| LS Z score | 1.50 ± 1.17 |
| FN BMD (g/cm$^2$) | 0.625 ± 0.127 |
| FN Z score | 0.32 ± 1.18 |
| 1/3R BMD (g/cm$^2$) | 0.517 ± 0.086 |
| 1/3R Z score | 0.37 ± 1.44 |
| BAP (U/L) | 31.8 ± 12.4 |
| Osteocalcin (ng/mL) | 7.3 ± 3.8 |
| Urine NTx (nMBCE/mM-Cr) | 52.6 ± 33.0 |
| Arms LBM (g) | 3550.8 ± 619.1 |
| Legs LBM (g) | 10,703.8 ± 2152.1 |
| RSMI (kg/m$^2$) | 6.33 ± 1.03 |
| OGN (pg/mL) | 83.8 ± 28.2 |
| FAM5C (ng/mL) | 3.04 ± 2.22 |

BMI, body mass index; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; T2DM, type 2 diabetes mellitus; LS, lumbar spine; BMD, bone mineral density; FN, femoral neck; 1/3R, one third of the radius; BAP, bone specific alkaline phosphatase; NTx, type I collagen cross-linked N-telopeptide; RSMI, relative skeletal muscle mass index; OGN, osteoglycin, FAM5C, family with sequence similarity 5, member C; LBM, lean body mass.

Serum OGN levels were measured by an ELISA kit (Cloud-Clone Corp., Houston, TX, USA) as indicated by the manufacturer. In brief, 96 wells of a microtiter plate were pre-coated with antibody specific to OGN. One hundred microliters of serum samples or standards was placed in each of the 96 wells with a biotin-conjugated antibody specific to OGN. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain OGN, biotin-conjugated antibody and enzyme-conjugated Avidun will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of OGN in the samples is then determined by comparing the OD of the samples to the standard curve. The minimum detective dose of this kit is typically <12.5 pg/mL. No significant cross-reactivity or interference between OGN and analogues was observed. The coefficient variation (CV) of measurements of OGN was 2.2%. Serum FAM5C levels were measured by another ELISA kit (USCN Life Science, Cologne, Germany) as indicated by the manufacturer. In brief, 96 wells of a microtiter plate were pre-coated with antibody specific to FAM5C. One hundred microliters of serum samples or standards was placed in each of the 96 wells with a biotin-conjugated antibody specific to FAM5C. Next, Avidin conjugated to HRP is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain FAM5C, biotin-conjugated antibody and enzyme-conjugated Avidun will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of FAM5C in the samples is then determined by comparing the OD of the samples to the standard curve. The detection range of this kit is 0.312–20 ng/mL. No significant cross-reactivity or interference between FAM5C and analogues was observed. The CV of measurements of FAM5C was 1.9%.