Investigation of bone mineral density and the changes by enzyme replacement therapy in patients with Fabry disease

Nose, Yuma ; Fujii, Hideki ; Goto, Shunsuke ; Kono, Keiji ; Okamoto, Hayaki ; Watanabe, Kentarou ; Nishi, Shinichi

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Highlights

- Bone mineral density in male patients with Fabry disease was lower than the averaged bone mineral density of their same age although it is preserved in female patients.
- Plasma lyso-Gb3 levels showed a significantly negative correlation with lumbar spine and femoral bone mineral density in male patients with Fabry disease.
- Enzyme replacement therapy ameliorated bone mineral density only in male patients with Fabry disease.

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Investigation of Bone Mineral Density and the Changes by Enzyme Replacement Therapy in Patients with Fabry Disease

Running title: Bone mineral density in Fabry disease

Authors: Yuma Nose, MD[1], Hideki Fujii, MD, PhD, FASN[1], Shunsuke Goto, MD, PhD[1], Keiji Kono, MD, PhD[1], Hayaki Okamoto, MD[1], Kentaro Watanabe, MD, PhD[1], Shinichi Nishi, MD, PhD[1]

Affiliations**:**

[1] Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, Kobe, Japan

Corresponding author:

Hideki Fujii, MD, PhD, FASN.

Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine

7-5-2, Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650-0017, Japan

TEL: +81-78-382-6500, FAX: +81-78-382-6509

E-mail: fhideki@med.kobe-u.ac.jp

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Author contributions

Y.N. wrote the text. F.H. revised it critically for important intellectual content. S.G. and K.K. analyzed the data. O.H., K.W., and K.S. interpreted the data. S.N. drafted this study. All co-authors reviewed and approved this paper.

Abstract

Background

Fabry disease (FD) is an inherited disorder that causes organ dysfunction. However, only a few studies have reported on bone mineral density (BMD) in FD patients, and the relationship between BMD and clinical factors such as globotriaosylsphingosine (lyso-Gb3) remains unclear. Therefore, the current study sought to investigate BMD in FD patients, the relationship between BMD and lyso-Gb3, and the effects of enzyme replacement therapy (ERT) on changes in BMD and lyso-Gb3.

Methods

This single-center, observational study included 15 patients who visited our facility for FD between January 2008 and June 2021. We assessed BMD and clinical characteristics in study patients, including plasma lyso-Gb3 levels, and examined the relationship between BMD and plasma lyso-Gb3 levels, and changes in BMD after starting ERT.

Results

Male patients' MBD had reduced, whereas female patients' BMD was preserved. Male patients had significantly higher plasma lyso-Gb3 levels than female patients. Moreover,

plasma lyso-Gb3 levels were found to be significantly related to the lumbar spine and femoral BMD. These were strongly linked with plasma lyso-Gb3 levels in male patients, whereas no strong link was observed in female patients. Furthermore, BMD significantly increased only in male patients although plasma lyso-Gb3 levels significantly decreased by ERT in all patients.

Conclusion

BMD decreased possibly due to Gb3 accumulation, and ERT could increase BMD in male FD patients.

Keywords:

bone mineral density, enzyme replacement therapy, Fabry disease, lyso-Gb3

1. Introduction

Fabry disease (FD) is an X-linked genetic disorder that manifests clinically as extremity pain, skin lesions, hypohidrosis, cardiac hypertrophy and dysfunction, proteinuria and kidney dysfunction, and cerebrovascular lesions. According to a recent study, the prevalence of FD was approximately 1 case per 7,000 newborns [1]. The genetic mutation of alpha-galactosidase A (GLA) is known to cause FD, and the accumulation of globotriaosylceramide (Gb3), which is a substrate of GLA, in systemic organs leads to the manifestation of clinical symptoms and organ disorders. Cardiomyocytes, vascular endothelium, podocytes, tubular cell, epithelial and sweat gland cell, and nerve cells have all been found to accumulate Gb3 [2-4]. Recently, it was reported that globotriaosylsphingosine (lyso-Gb3), a derivative of Gb3, is a more specific biomarker for FD than Gb3, and is significantly elevated in FD patients with severe symptoms [5]. Many studies found that FD treatment, including enzyme replacement therapy (ERT), chaperone therapy, and gene therapy, can lower plasma Lyso-Gb3 levels [6-8].

Although there have been numerous reports of organ involvement in FD patients, only a few studies have reported bone involvement in those with FD [9-11]. Furthermore, no studies assessed the relationship between bone mineral density (BMD) and lyso-Gb3, or the effect of FD treatment on BMD.

Thus, our study sought to investigate the relationship between BMD and lyso-Gb3 as well as the effect of ERT on BMD in patients with FD.

2. Method

2.1. Study design and population

This study was a retrospective observational study that included consecutive 24 FD patients (10 males and 14 females) who visited our department between January 2008 and June 2021. All patients had their GLA activity reduced and underwent a genetic analysis of GLA gene mutations to diagnose FD. We excluded five patients who had no BMD data and four patients who had the E66Q mutation, which is a functional polymorphism [12]. After these exclusions, the remaining 15 patients were included in this study (7 males and 8 females). To analyze the effect of ERT, we evaluated changes in BMD in patients who had baseline data before starting ERT and at 2 years. This research was conducted following the Helsinki Declaration. Our protocol was authorized by the Institutional Review Board of Kobe University Graduate School of Medicine (No. B210251).

2.2. Data and sample collection

From medical records, relevant clinical information including clinical characteristics such as age, gender, past medical history, and blood and urine laboratory findings were obtained. The estimated glomerular filtration rate (eGFR) was determined using the Japanese GFR estimation formula [13, 14].

2.3. Measurement of BMD

BMD was determined using Discovery® (Hologic, Bedford, MA, USA) or Horizon A® (Hologic, Bedford, MA, USA) dual-energy bone X-ray absorptiometry. Cross-calibration procedures were performed on two devices. Using a standard protocol, we assessed the mean values of L2-L4 as lumbar spine BMD and total hip as femoral BMD. We used Z-score for analysis in this study because it was proposed to be used for BMD assessment in young patients [15]. The software reference data were used to generate Z-scores for lumbar spine and femoral BMD in adults and lumbar spine BMD in young patients. The previous studies provided reference data for femoral in children [16-18]. The measurement was conducted at baseline and two years after starting ERT.

2.4. Measurement of GLA activity and plasma lyso-Gb3 levels

GLA activity in leukocytes was assessed by fluorescence method using 4-Methylumbelliferyl-α- D-galactoside, N-Acetyl-D-galactosamine.

Plasma lyso-Gb3 level was measured by liquid chromatograph-tandem mass spectrometry (LC-MS/MS). Xevo TQ-XS (Waters, Milford, Massachusetts, USA) was employed as a mass spectrometer and ACQUITY UPLC I-Class Systems (Waters, Milford, Massachusetts, USA) as a liquid chromatography system.

2.5. Statistical analysis

Statistical analysis was conducted using IBM SPSS statistics version 27.0 (SPSS Inc., Illinois, USA). Continuous variables were depicted as mean ± standard deviation. We used t-tests, the Mann-Whitney test, and Fisher's exact test to compare patient characteristics and BMD at baseline, as well as changes in BMD before and after ERT in men and women. The paired t-test was used to compare BMD and lyso-Gb3 levels before and after ERT. Pearson's correlation analysis was performed to investigate the relationship between plasma lyso-Gb3 levels and BMD. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Patient characteristics

Table 1 displays the overall, male and female patients' characteristics at baseline. None of the patients had diabetes mellitus. Females were significantly older than males, and their eGFR was significantly lower than in males. Males had significantly lower GLA activity and significantly higher plasma lyso-Gb3 levels than females. Among the 15 study patients, 2 patients had already started ERT. As for patients who had never received ERT, α-galactosidase A (agalsidase β, Fabrazyme®; Genzyme Corp., Cambridge, MA, USA or agalsidase α, Replagal®; Shire plc, Dublin, Ireland) was given intravenously every 2 weeks (at a dosage of 1 mg/kg body weight or 0.2 mg/kg body weight, respectively) for 6 and 7 patients, respectively.

Patients with the following four mutations were included in this study; c.1124G>A (N = 1), c.928C>T (N = 7), c.281G>A (N = 2) and c.658C>T (N = 5). Although the number of study patients was small, there was no significant correlation between the genetic variants and BMD.

3.2. Comparison of BMD between male and female patients

Figure 1 depicts BMD Z-scores in male (N = 7) and female patients (N = 8). Males had significantly lower lumbar spine and femoral BMD Z-scores than females (lumbar spine

159 Z-score: -1.9 ± 2.0 versus 0.8 ± 0.8, $P < 0.05$; femoral Z-score: -1.0 ± 1.4 versus 0.6 ± 0.6,
160 $P < 0.05$). Furthermore, male BMD was lower than the average BMD of the same age,
161 whereas female BMD was higher than the average BMD of the same age. The Z scores
162 were significantly and positively correlated with age among all the study patients
163 (lumber spine, r = 0.68, $P < 0.01$: femoral, r = 0.70, $P < 0.01$, respectively). However, a
164 significant correlation was observed only in male patients.
165

166 **3.3. Correlation of plasma lyso-Gb3 levels with BMD**

167 Plasma lyso-Gb3 levels correlated negatively with both lumbar spine and femoral
168 BMD Z-scores (lumbar spine, r = -0.85, $P < 0.01$: femoral, r = -0.83, $P < 0.01$, respectively;
169 Figure 2A). Furthermore, in each gender, we assessed the link between plasma lyso-Gb3
170 levels and lumbar spine and femoral BMD Z-scores in each gender (Figure 2B). In male
171 patients, plasma lyso-Gb3 levels were significantly and negatively correlated with both
172 lumbar spine and femoral BMD Z-scores (lumbar spine: r = -0.92, $P < 0.01$; femoral: r = -
173 0.91, $P < 0.01$, respectively), but not in female patients (lumbar spine: r = 0.57, $P = 0.18$,
174 femoral: r = 0.01, $P = 0.99$, respectively).
175

176 **3.4. Changes in plasma lyso-Gb3 levels and BMD before and after starting ERT**

After starting ERT, plasma lyso-Gb3 levels were significantly reduced in all patients, with males experiencing a greater reduction than females (Figure 3). Among 15 patients, two who had received ERT, two who took osteoporosis medication, and one who did not have BMD data at 2 years were excluded from the BMD change analysis. Males had greater changes in BMD Z-scores in a year (ΔZ-score) of the lumbar spine after starting ERT (0.9 ± 0.9 versus -0.2 ± 0.2, $P < 0.05$, Figure 4A) than females. Males had a greater femoral ΔZ-score than females, though there was no statistically significant difference (0.5 ± 0.8 versus. -0.2 ± 0.3, $P = 0.15$, Figure 4B). Furthermore, a significant relationship between Δlyso-Gb3 and ΔZ-score of femoral was found in males ($r = 0.95$, $P < 0.05$, Figure 5).

4. Discussion

Our study demonstrated that: 1) BMD Z-scores of the lumbar spine and femoral were considerably lower in males than in females; 2) BMD in males was lower than the averaged BMD of their age; 3) plasma lyso-Gb3 levels showed a significantly negative correlation with lumbar spine and femoral BMD in males; and 4) although ERT reduced plasma lyso-Gb3 levels in all patients, it only improved BMD in males.

Although many studies reported cardiac, kidney, and cerebrovascular involvement in FD patients, only a few studies have reported skeletal involvement. The majority of FD male patients reportedly had decreased BMD, whereas premenopausal female patients did not [9-11, 19]. Furthermore, these studies did not examine the relationship between biomarkers related to FD like Gb3 and lyso-Gb3, and skeletal lesions. In line with the findings of these studies, our study found that BMD was reduced only in male patients. Although we compared the BMD of premenopausal female patients to that of postmenopausal female patients in this study, no significant difference was found.

Several pathophysiological mechanisms for decreased BMD in FD have been proposed. One of them is the use of antiepileptic drugs, which are thought to affect bone metabolisms [11, 20, 21]. Carbamazepine, phenobarbital, valproate, oxcarbazepine, and gabapentin, etc. have been linked to decreased vitamin D absorption and utilization, As well as adverse effects on hormones such as parathyroid hormone, estrogens, and calcitonin [20, 21]. Because the majority of male FD patients have severe pain and take medication to alleviate it, the influence should be considered. In our study, however, only one male patient took carbamazepine. Therefore, we assumed that its impact on bone metabolism could be exempted. Furthermore, it is well-known that factors related to kidney dysfunction-related such as uremic toxins accumulation, taking multiple

212 medications, mineral bone disorder, and the activation of the renin-angiotensin-
213 aldosterone system and sympathetic nerve system all contribute to the development of
214 bone abnormalities. Because previous studies included patients with kidney dysfunction,
215 factors associated with decreased kidney function may influence a reduction in BMD.
216 Our study, however, excluded those with severe kidney dysfunction. Furthermore, FD
217 patients are more likely to stay at home and avoid sunlight exposure due to extreme
218 pain and hypohidrosis. These factors may contribute to a reduction in BMD and vitamin
219 D deficiency. Vitamin D deficiency or insufficiency was found in all the study patients in
220 our study (vitamin D deficiency, N = 11: vitamin D insufficiency, N = 4). An experimental
221 study found that FD model mice did not have vitamin D deficiency and that secondary
222 hyperparathyroidism due to tubular impairment caused an acceleration of bone
223 resorption and osteomalacia due to hyperphosphaturia and hypercalciuria, resulting in
224 low BMD [22]. Gb3 accumulates in various organs in FD patients, causing organ damage.
225 Chemical analysis of the sphingolipid extracted from the femoral head revealed the
226 presence of Gb3 [23]. Furthermore, the study also reported that the result of the
227 immunohistochemical analysis revealed that Gb3 accumulated predominantly in
228 endothelial cells, osteocytes, and chondrocytes in FD patients. Therefore, Gb3
229 accumulation in bone tissues, as well as impaired microcirculation to bone tissues due

to Gb3 accumulation in vascular endothelial cells, may contribute to the progression of bone lesions. In our study, plasma lyso-Gb3 levels were found to be significantly correlated with BMD in male FD patients. In general, male FD patients have significantly higher plasma lyso-Gb3 levels than female FD patients, implying a significant accumulation of Gb3 in bone tissues. Taken together, although a variety of factors may contribute to the development of bone abnormalities in FD, we believe that the accumulation of Gb3 may contribute to a decrease in BMD.

Several previous studies have found that ERT can lower plasma lyso-Gb3 levels and partially eliminate Gb3 in organs and tissues [6, 24-27]. Accordingly, it is thought that ERT can also reduce Gb3 accumulation in bone tissues. ERT also reduced plasma lyso-Gb3 levels, particularly in male FD patients, according to our findings. Furthermore, BMD was improved, and changes in plasma lyso-Gb3 were related to changes in BMD in male patients. Therefore, it is suggested that ERT could be a crucial treatment to maintain bone health in male FD patients.

There are several limitations to this study. First, the number of patients included in this study was relatively small. However, as well-known, because FD is a rare disease, it is difficult to gather a large number of patients at a single center. We intend to conduct a similar study with large cohort data in the near future. Second, the characteristics of

248 male and female patients differed slightly. Female patients were significantly older than
249 male patients and had significantly lower eGFR. Despite these characteristics, female
250 patients' BMD was not reduced. Male patients, on the other hand, had lower BMD
251 despite being young and having normal kidney function. Therefore, osteopenia is
252 thought to be a significant complication in male FD patients. Third, as previously stated,
253 a few studies have already reported on osteopenia in male FD patients [9-11, 19]. Unlike
254 previous studies, however, ours examined not only the relationship between lyso-Gb3
255 and BMD but also the effect of ERT on BMD. We thought these points were significant
256 and unique to the current study.

257 In conclusion, our findings suggested that lumbar spine BMD and femoral BMD
258 decreased in male FD patients, possibly due to lyso-Gb3 accumulation in bone tissue,
259 and that ERT could improve BMD in male FD patients.
260

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Table 1.

	All patients (N = 15)	Male (N = 7)	Female (N = 8)	*P*
Age (year)	37 ± 20	19 ± 5	54 ± 12	< 0.05
ERT (%)	2 (13)	1 (14)	1 (13)	1.00
Corrected Ca (mg/dL)	9.5 ± 0.4	9.4 ± 0.3	9.5 ± 0.5	0.34
P (mg/dL)	3.8 ± 0.7	3.8 ± 0.9	3.7 ± 0.5	0.81
eGFR (mL/min/1.73 m^2)	90 ± 31	120 ± 16	65 ± 12	< 0.05
uACR (mg/g・Cr)	390 ± 719	53 ± 76	685 ± 903	0.40
u-pro (g/g・Cr)	0.6 ± 1.1	0.1 ± 0.1	1.0 ± 1.4	0.61
GLA activity (nmol/mg protein/h)	23.3 ± 27.8	0.1 ± 0.1	43.3 ± 23.3	< 0.05
Lyso-Gb3 (ng/mL)	87 ± 86	167 ± 54	16 ± 15	< 0.05

ERT, Enzyme replacement therapy; eGFR, estimated glomerular filtration rate; uACR, urine albumin-creatinine ratio; u-pro, urinary protein; GLA, alfa-galactosidase A; Lyso-Gb3, globotriaosylsphingosine.

Values are presented as the mean ± SD.

406 **Figure legends**

407 Figure 1. Differences in BMD between male and female patients with FD

408

409 A. BMD Z-score of the lumbar spine

410 B. BMD Z-score of femoral

411

412 BMD, bone mineral density; FD, Fabry disease.

413 *; $P < 0.05$

414

415 Figure 2. Correlation between lyso-Gb3 and lumber and femoral BMD

416

417 A. BMD Z-score of the lumbar spine and femoral in all patients

418 B. BMD Z-score of the lumber spine and femoral in each gender

419

420 lyso-Gb3, globotriaosylsphingosine; BMD, bone mineral density.

421

422 Figure 3. Changes in plasma lyso-Gb3 levels after starting ERT

423

A. All patients

Excluded two patients who had received ERT

B. Male patients

C. Female patients

lyso-Gb3, globotriaosylsphingosine; ERT, enzyme replacement therapy.

*; $P < 0.05$

Figure 4. Changes in the lumbar spine and femoral BMD after starting ERT

A. Changes in BMD Z-score of the lumber spine and femoral in males

B. Changes in BMD Z-score of the lumbar spine and femoral in females

BMD, bone mineral density; ERT, enzyme replacement therapy.

*; $P < 0.05$

Figure 5. Association between changes in lyso-Gb3 and those in BMD in each gender

442 A. Male patients

443 B. Female patients

444

445 lyso-Gb3, globotriaosylsphingosine; BMD, bone mineral density.

Figure 1

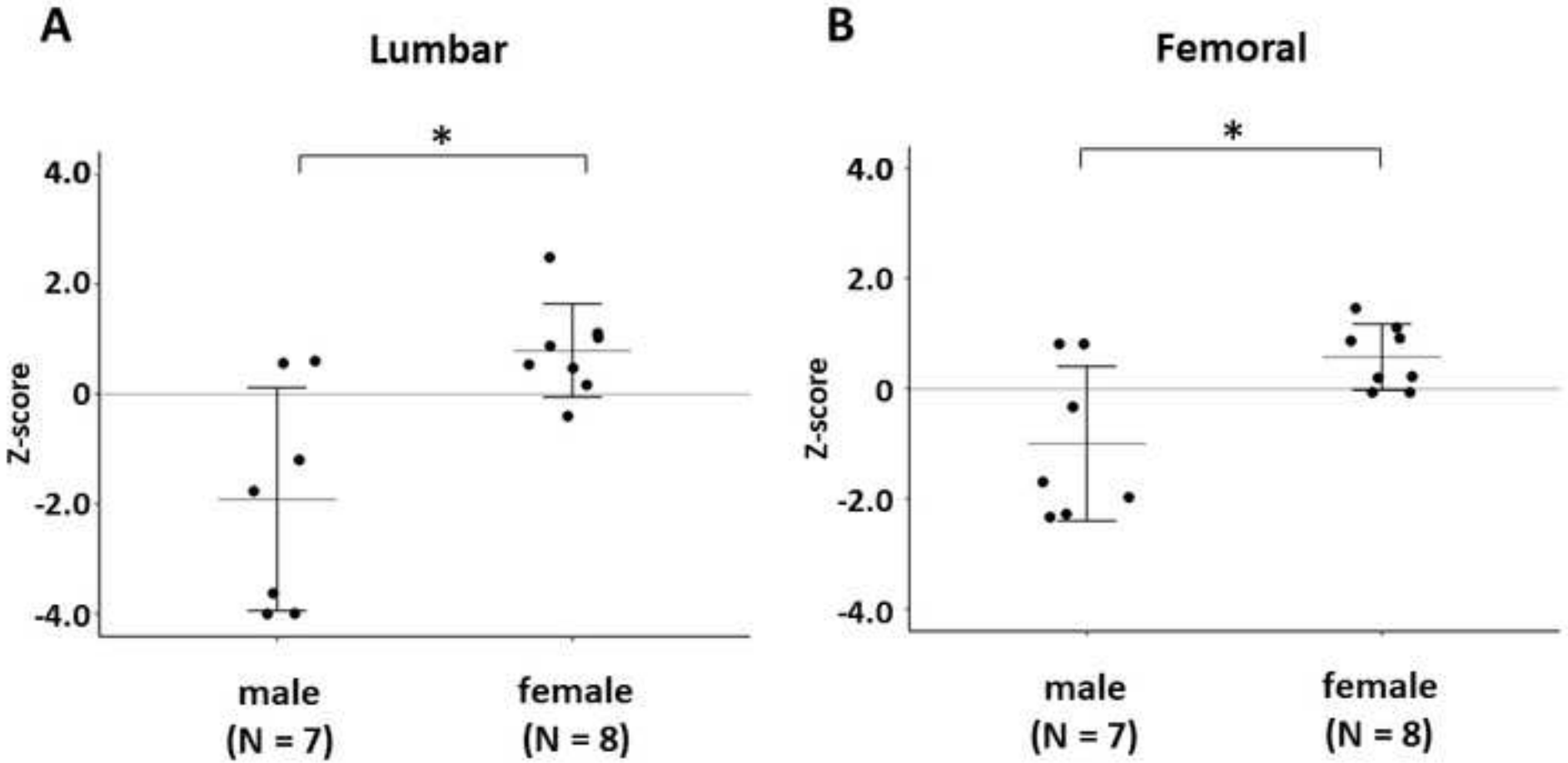

Figure 2A

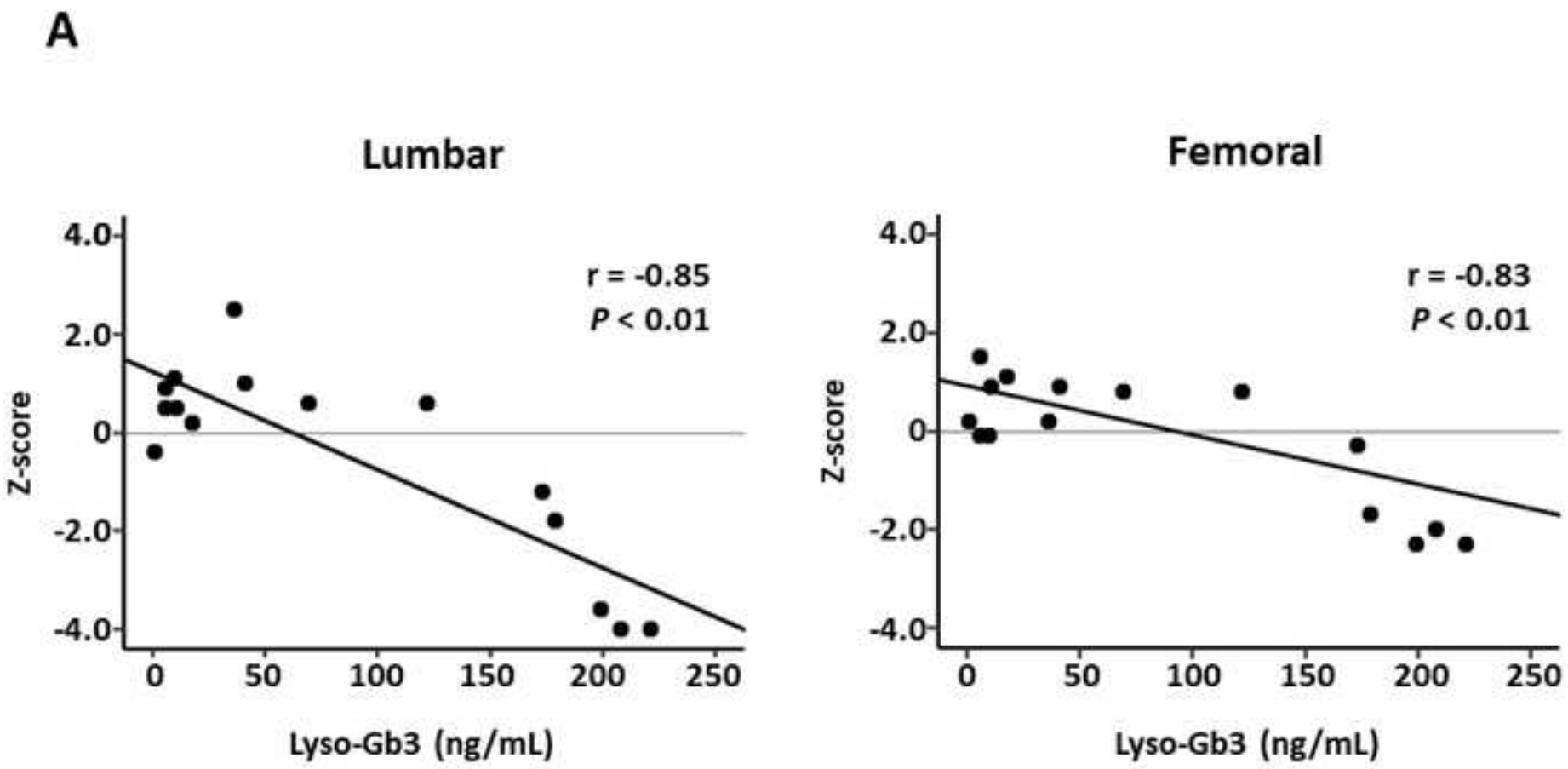

Figure 2B

Click here to access/download;Figure;lysoGb3 bone (Figure 2B).tif

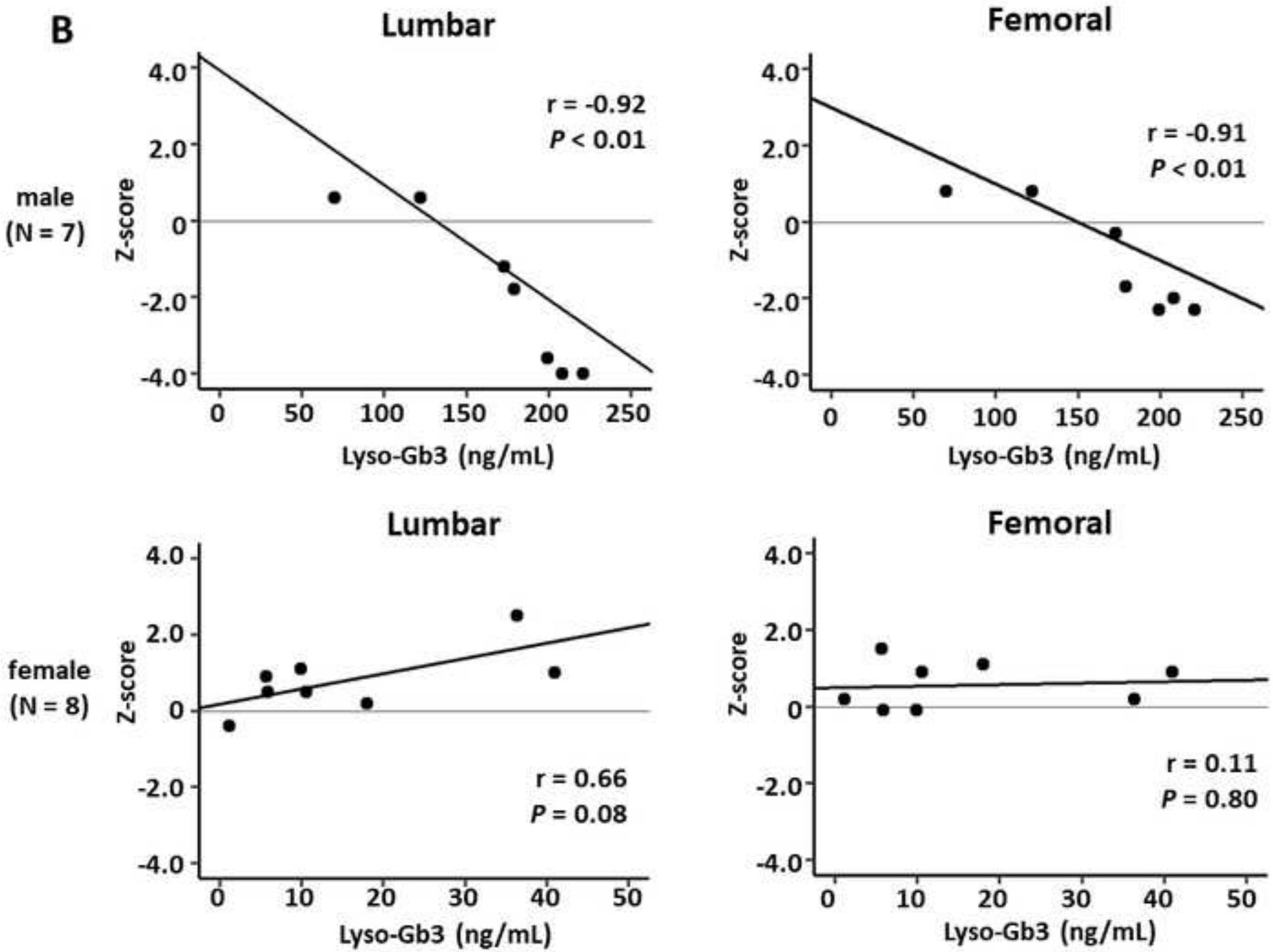

Figure 3

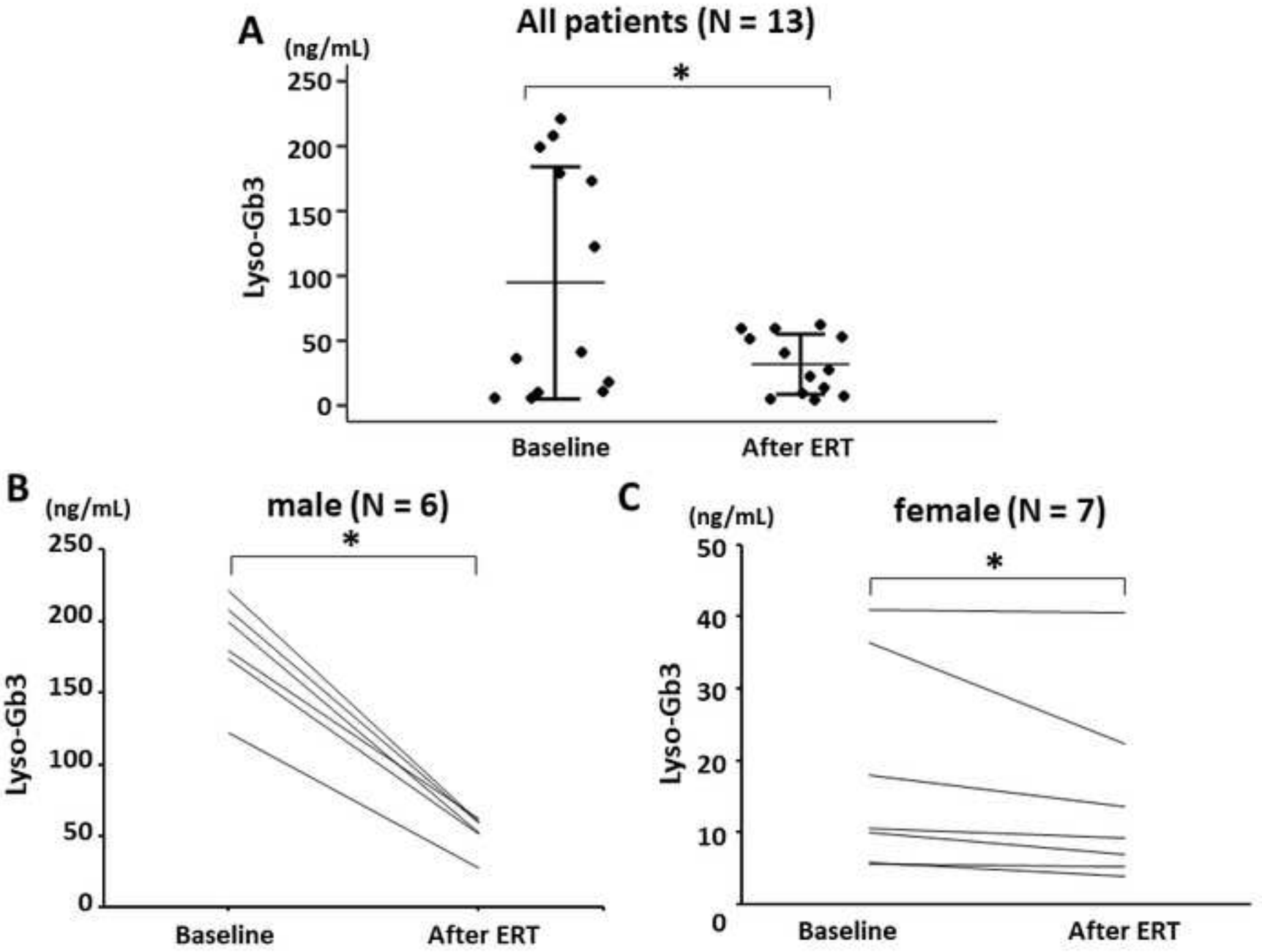

Figure 4

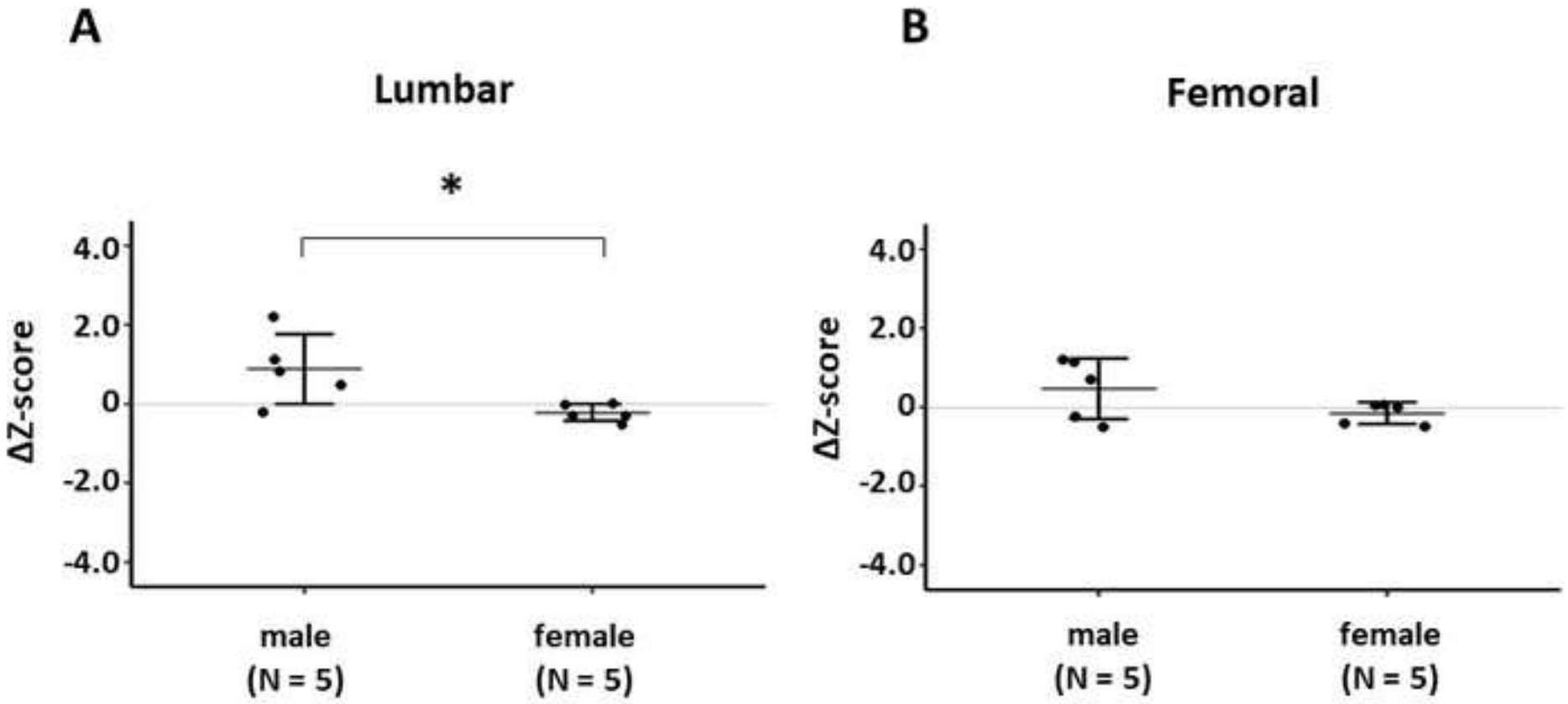

Figure 5