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Original Article

Plasma Growth and Differentiation Factor 15 Predict Longitudinal Changes in Bone Parameters in Women, but Not in Men

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Abstract

Bone fragility can progress with aging, but biomarkers to detect emerging osteopenia have not been fully elucidated. Growth/differentiation factor 15 (GDF-15) has pleiotropic roles in a broad range of age-related conditions, but its association with osteopenia is unknown. We examined the relationship between plasma GDF-15 levels and rate of change in bone parameters over 9 years of follow-up in 596 adults in the InCHIANTI study (baseline age, 65–94 years; women, 52.4%; mean follow-up, 7.0 ± 3.0 years). Plasma GDF-15 concentrations were measured using the 1.3k HTS SOMAscan assay. Eight bone parameters were measured in the right tibia by peripheral quantitative computed tomography; total bone density, trabecular bone density, medullary plus trabecular bone density, cortical bone density, total bone area, cortical bone area, medullary bone area, and minimum moment of inertia (mMOI). We ran sex-specific linear mixed-effect models with random intercepts and slopes adjusted for age, age-squared, education, body mass index, the rate of change in weight, smoking, sedentary behavior, cross-sectional areas of calf muscles and fat, 25-hydroxyvitamin D, parathyroid hormone, calcium, diabetes mellitus, and follow-up time. We found a significant association of "baseline GDF-15 × time" in models predicting cortical bone density and the mMOI in women, suggesting that the rates of decline in these bone parameters increased with higher GDF-15 (false discovery rate <0.05). Higher plasma levels GDF-15 predicted an accelerated decline in bone parameters in women, but was less associated in men. Furthermore studies are needed to understand the mechanisms underlying these sex differences.

Keywords: Cortical bone, Growth/differentiation factor 15, Osteopenia, Sex difference, Trabecular bone

A large body of evidence from diverse populations has consistently shown that osteopenia (ie, decline in bone density) is associated with an elevated risk of developing a wide range of adverse health events, including osteoporotic fractures, severe disability, and death (1,2). With the aging of the population, the number of persons afflicted with osteoporosis has grown worldwide, with the International Osteoporosis Foundation reporting that the number reached over 200 million (3). The prevalence of osteoporosis increases with age in both men and women, with 1 in 2 women and 1 in 5 men aged more than 50 years developing 1 or more osteoporotic fractures in their lifetime (4–6). Medications, nutrition, and exercise interventions have been shown to increase bone mineral density (BMD) in osteoporotic patients (7,8). However, once the diagnosis of osteoporosis is established it is challenging to restore bone quality and quantity to a normal level even with years of pharmacological and nonpharmacological intervention (9,10). Identification of factors

associated with osteoporosis and the risk of developing osteoporosis could provide insight towards developing effective new treatments.

Fibroblast growth factor and transforming growth factor (TGF)-β were found to reduce bone loss in experimental animal models of osteoporosis, and reduced expression of TGF-β in bone was found in several animal models of osteopenia (11).

Growth/differentiation factor 15 (GDF-15) is a 25 kDa protein coded by *GDF15* on chromosome 19(p13.11). It is distantly related to the TGF-β superfamily that is highly expressed in response to tissue injury in the liver, kidneys, and heart (12). GDF-15 is a core member of the senescence-associated secretory phenotype (SASP) and considered a marker of systemic energy homeostasis (13). The pleiotropic biological roles of GDF-15 are still controversial, particularly its regulatory role in inflammation (12,14,15). Our previous study showed that higher plasma GDF-15 levels were associated with chronological age and predicted mobility disability over 9 years of follow-up (16,17). This suggests that GDF-15 plays a role in conditions that have a negative impact on mobility, such as degradation of muscle, bone, and joints. Whether GDF-15 plays a role in the development of bone tissue pathology has not been fully elucidated. GDF-15 is secreted by osteocytes under hypoxic conditions and circulating GDF-15 levels positively correlate with osteoclast activity markers, but negatively correlate with osteoblast activity markers in vitro (18,19). Cross-sectional studies demonstrated a negative association between serum GDF-15 and lumbar spine, femoral neck, and total hip BMD in postmenopausal Asian women, but longitudinal studies are lacking (20,21).

BMD is generally measured at the femoral neck or spine using dual-energy X-ray absorptiometry (DXA). However, BMD measurements by DXA have several limitations (22,23): (a) BMD only provides 60% of variation in bone fragility (24); (b) mean values of BMD are based on 2-dimensional assessments (areal BMD) and are largely affected by bone size; (c) BMD does not differentiate among cortical, trabecular, and medullary bone; and (d) BMD measured by DXA is influenced by soft tissues around the bone. Peripheral quantitative computed tomography (pQCT) overcomes most of these limitations in bone parameter assessment. It measures volumetric BMD in the appendicular skeleton, cross-sectional area of bones, and minimum moment of inertia (mMOI), indicating bone strength. Because it also detects relatively small changes in bone and soft tissue (25), pQCT may help strengthen our understanding of longitudinal changes in the appendicular skeleton. Epidemiological studies with pQCT have demonstrated that age-related bone loss is heterogeneous among different bone tissues such as cortical and trabecular bone and is also quite different between women and men (22). Nevertheless, there are several important gaps in knowledge about the relationship between GDF-15 and bone tissue. First, no study has yet examined the association between GDF-15 and multiple bone parameters such as trabecular and cortical bones. Osteopenia progresses differently in different skeletal regions: early bone loss occurs in trabecular bone and later bone loss can be detected in cortical bone (26,27). Assessment of multiple bone-related parameters may give greater insight into the mechanisms by which GDF-15 may be linked to age-related changes in bone. Second, no longitudinal study has examined the association of GDF-15 with bone parameters in either sex. Some risk factors for osteoporosis are sex-specific, and it is therefore possible that GDF-15 identifies different longitudinal changes in bone parameters in men and women. We investigated whether, independently of major confounders, plasma GDF-15 levels predict the rate of change in bone parameters over time, and whether the changes differ by sex in community-dwelling adults aged ≥65 years.

Method

Study Population and Design

This longitudinal study used data from the Invecchiare in Chianti, "aging in the Chianti area" (InCHIANTI) study, a prospective cohort study aimed at investigating the aging process and identifying mechanisms that underlie decline in physical function and mobility with age (28). The InCHIANTI study measured bone-related parameters by pQCT at baseline, and at 3-year, 6-year, and 9-year follow-up visits (maximum, 4 visits). In our analysis, we selected 596 women and men ≥65 years (65–94 years; women, 52.4%) who were not taking hormonal or bisphosphonate therapy. We collected data on plasma GDF-15 and bone parameters as well as body mass index (BMI), years of education, smoking status, physical activity level, cross-sectional areas of calf muscles and fat, selected serum biomarkers, and disease status at the baseline visit (Figure 1). Mean follow-up was 7.0 ± 3.0 years. The total number of observations (person-visits) was 2 383 (1 247 for women and 1 136 for men). The study protocols complied with the Declaration of Helsinki and were approved by the Italian National Institute of Research and Care on Aging Ethical Committee, Ancona (Baseline, protocol #14/CE, February 28, 2000) and by the Local Ethical Committee at Azienda Sanitaria of Florence (follow up, protocol #5/04, May 12, 2004).

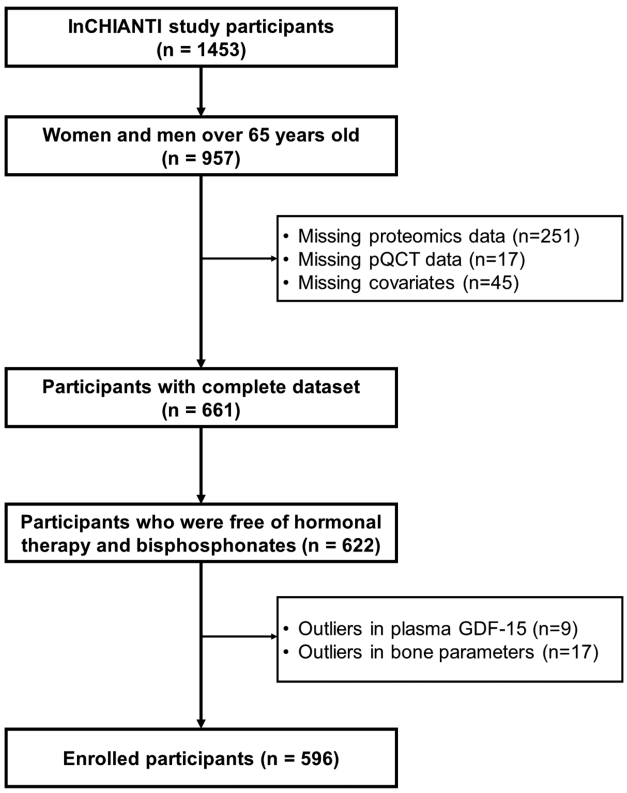

Figure 1. Flow diagram of included and excluded participants. GDF-15 = growth and differentiation factor 15; InCHIANTI = aging in the Chianti area; pQCT = peripheral quantitative computed tomography.

Written informed consent was obtained from all participants after an extensive description of the study.

Measurement of Plasma GDF-15

Blood samples for GDF-15 were collected in the early morning after an overnight fast. All samples were stored at 4°C, centrifuged within 4 h, then immediately divided into aliquots and frozen at −80°C. We measured plasma GDF-15 at baseline using the 1.3k HTS SOMAscan assay (SomaLogic, Inc., Boulder, CO) (29,30). GDF-15 was expressed in relative fluorescence units (RFU) and was normalized according to the Trans-NIH Center for Human Immunology, Autoimmunity, and Inflammation pipeline. Data normalization procedures have been described elsewhere (29). The overall technical variability of the assay is low (median intraplate coefficient of variation [CV] in the 3%–4% range). Our group has previously examined the correlation between 2 plasma GDF-15 levels using an aptamer-based assay and ELISA (r = 0.82) (16). We excluded GDF-15 data above/below 3 standard deviations (*SD*) from the mean value as outliers.

Bone Parameters

In the InCHANTI study, pQCT (XCT 2000, Stratec Medizintechnik, Pforzheim, Germany) was used to measure bone parameters of the right tibia. Detailed testing procedures have been previously reported (22). The length of the tibia was measured as the distance from the medial knee joint cleft to the medial malleolus. Standard transverse scans were obtained at 4% (proximal), 38%, and 66% (from the distal end) points along the tibial length. The image data were analyzed using commercial software (BonAlyse, BonAlyse Oy, Jyvaskyla, Finland). The following 8 parameters were analyzed:

(1) Total bone density (mg/cm^3): mean density of an axial section measured at 4% of the total tibial length from the medial malleolus.

(2) Trabecular bone density (mg/cm^3): mean density of the trabecular bone (centroid that covers 50% of the total area) measured at 4% of the total tibial length from the medial malleolus.

(3) Cortical bone density (mg/cm^3): mean density of the cortical bone measured at 38% of the total tibial length from the medial malleolus.

(4) Medullary and trabecular bone density (mg/cm^3): mean density of the medullary and trabecular bone area measured at 38% of the tibial length from the distal end.

(5) Cross-sectional total bone area (mm^2): total area, including cortical and medullary areas. This measurement was obtained at 38% of the tibial length from the distal end using a threshold of 180 mg/cm^3 to separate bone from surrounding soft tissues.

(6) Cross-sectional cortical bone area (mm^2): cross-sectional area of cortical bone at 38% of the tibial length from the distal end using a threshold of 710 mg/cm^3 to distinguish cortical from medullary bone; the area was within the internal and external merging of the cortical ring.

(7) Cross-sectional medullary area (mm^2): medullary area was measured at 38% of the tibial length from the distal end as the difference between total and cortical bone areas. It includes the marrow space and areas of the inner cortex trabecularized by endocortical resorption that have a cortical apparent bone density <710 mg/cm^3.

(8) Minimum moment of inertia (mMOI; g/cm): the moment of inertia was obtained as a density-weighted moment of inertia at 38% of the tibial length from the distal end. The mMOI provides an estimate of the minimal resistance to bending and reflects cortical bone density and bone geometric distribution (31).

Covariates

Adjustment covariates collected at the baseline visit were age, years of education, BMI, smoking status, physical activity, history of hip fracture, cross-sectional areas of calf muscles and fat, serum vitamin D (25 [OH]D), intact parathyroid hormone (PTH), and calcium, chronic disease status (metabolic syndrome, coronary heart disease, stroke, cancer, diabetes, congestive heart failure, and chronic obstructive pulmonary disease), and follow-up time (year). Serum level of vitamin D (25[OH]D, nmol/L) was measured by radioimmunoassay (RIA kit; DiaSorin, Stillwater, MN). Intraassay and interassay CVs for vitamin D were 8.1% and 10.2%, respectively. Serum PTH (ng/L) levels were measured with a 2-site immunoradiometric assay kit (N-tact PTHSP; DiaSorin). Intraassay and interassay CVs for PTH were <3.0% and 5.5%, respectively. Total serum levels of calcium (mg/dL) were measured using a colorimetric assay with endpoint determination and sample blank (Roche Diagnostics, GmbH, Mannheim, Germany) and a Roche analyzer (Roche Diagnostics). Intraassay and the interassay CVs for calcium were 0.9% and 1.5%, respectively. Physical inactivity was defined as a participant reporting "hardly any physical activity" or "mostly sitting/some walking" in the last year. Cross-sectional areas of the calf muscles and fat at 66% of the tibial length from the distal end were measured by pQCT. Because body weight is associated with both bone loss and GDF-15 levels, and since body weight decreased over time in our samples (Overall change per year, β = −0.19, standard error [*SE*] = 0.03, p < .0001 from a linear mixed-effect model), each individual's rate of change in body weight (kg/y) was used as a covariate in our analysis. Diabetes mellitus was defined as meeting at least one of the following criteria: a physician's diagnosis in the medical history, current treatment with insulin or oral hypoglycemic drugs, self-report of diabetes, and measured fasting blood glucose level of ≥7.77 nmol/L.

Statistical Methods

Descriptive data are shown as mean ± *SD* for continuous variables that were normally distributed (histogram, QQ plots, and Shapiro-Wilk test); median value (interquartile) for continuous variables that deviated from the normal distribution; and percentages for categorical variables. For skewed data, we performed log-transformation before the primary analysis. Outliers were defined as 3 *SD* from mean values and were excluded from analysis. For comparisons by sex and baseline GDF-15 level (women, 1 840.5 RFU; men, 2 016.4 RFU), we performed age-adjusted linear regression models for continuous variables and age-adjusted logistic regression models for categorical variables. To test whether circulating levels of GDF-15 at baseline were associated with accelerating/decelerating changes in bone parameters, we performed linear mixed-effect models with each bone parameter as the dependent variable and baseline GDF-15 and its interaction with follow-up time (years) as the independent variable. In our preliminary analyses, we examined the association between chronic disease status described in the *Other Covariates* and the rate of change in bone parameters and found that multimorbidity status (0–7) and diabetes mellitus were consistently associated with the rate of change in bone parameters. Additionally, we examined the association between sex and rate of change in bone parameters by introducing a 3-way interaction term (sex × time × GDF-15) and three 2-way interaction terms (sex × time; sex × GDF-15; time × GDF-15). When we introduced the 3-way interaction term, we did not find a significant interaction mainly due to insufficient power (Supplementary Table 1). In models including only these

2-way interaction terms, except mMOI, the interaction term of sex × time was consistently significant, suggesting that the rate of change in bone parameters differs by sex (Supplementary Table 2).

For easier interpretation of our findings, we fitted sex-specific linear mixed-effect models. Specifically, we performed sex-specific models including a 2-way interaction (ie, time × GDF-15) to test whether changes in bone parameters were significantly associated with baseline GDF-15 levels. Our model included the following fixed effects: age centered at 70 years, level of education, BMI, the individual rates of change in body weight (kg/y) estimated from a linear mixed-effect model, smoking status, physical activity, cross-sectional areas of calf muscles and fat, 25 (OH)D, PTH, calcium, diabetes mellitus, follow-up time (year), and random effects: intercept and slope, with unstructured covariance. Random effects allowed individual-specific levels of bone parameters and rates of change to vary. Variance inflation factors among independent variables were <4. To visualize the interaction term, after splitting the participants into 2 groups using sex-specific median values of GDF-15 (women, 1 840.5 RFU; men, 2 016.4 RFU), the least-squares mean values of bone parameters were obtained from linear mixed-effect models adjusted for the covariates described earlier. SAS version 9.4 for Windows (SAS Institute, Inc., Cary, NC) was used for all data processing and statistical analyses. We set the level of statistical significance at $p < .05$ (2-sided) for baseline comparisons and the false discovery rate (FDR) at <0.05 (2-sided) for adjusting multiple comparisons in the association between GDF-15 and rate of change in bone parameters.

Results

Baseline characteristics of the study participants are presented in Table 1. Men were more likely to be smokers than women, have higher education levels, greater physical activity, lower BMI, larger cross-sectional calf muscle area, smaller cross-sectional area of calf fat, higher GDF-15 levels, and overall better bone parameters except medullary plus trabecular bone density and medullary bone area ($p < .05$). Baseline characteristics of the study participants stratified by sex-specific median values of GDF-15 are also presented (Table 2). At baseline in women, those with higher GDF-15 levels were older and more likely to have diabetes mellitus ($p < .05$). In men, those with higher GDF-15 levels were older and were more likely to be smokers, inactive, and to have diabetes mellitus ($p < .05$).

In both sexes, our analysis detected a significant increase with age in cross-sectional area of total bone and medullary bone area (FDR < 0.0001), while other bone parameters significantly declined over time (FDR < 0.0001; Supplementary Table 1). No longitudinal changes were observed in the mMOI (FDR: 0.08 for women, 0.41 for men, respectively).

In women, GDF-15 was associated with an accelerated decline in total, trabecular, and cortical bone density, and the mMOI in nominal p values ($p < .05$); however, after multiple comparison adjustments, cortical bone density and the mMOI remained significant (FDR < 0.05; Table 3). In men, the association between GDF-15 and rates of change in total bone density, cortical bone area, and the mMOI were weak and no longer significant after multiple comparison adjustment ($p < .05$; FDR > 0.05).

Supplementary Figure 1 shows the estimated rate of change in bone parameters. Compared to men, women had either lower BMD or smaller bone area, and the decrease in BMD was steeper. In total BMD, a parallel decrease was observed in men, although total BMD values at baseline were higher in those with higher GDF-15 than in those with lower GDF-15. In contrast, in women, a sharper decrease was observed in those with higher GDF-15 compared to those with lower GDF-15. Similar trends were observed in trabecular and cortical bone densities.

Table 1. Participant Characteristics

	Overall (n = 596)	Women (n = 312)	Men (n = 284)	p*
Age (y)	73.2 ± 6.1	73.6 ± 6.2	72.7 ± 6.0	.07
Follow-up time (y)	7.0 ± 3.0	6.9 ± 3.0	7.1 ± 2.9	.97
Sex (women, %)	52.4	—	—	—
Education (≥6 y, %)	28.2	16.0	41.6	<.0001
Current smoker (%)	15.1	7.7	23.2	<.0001
Inactive (%)	13.6	18.6	8.1	.001
Diabetes (%)	11.9	11.2	12.7	.60
Body mass index (kg/m^2)	27.5 ± 4.1	28.1 ± 4.6	26.9 ± 3.3	.0003
Annual rate of change in body weight (kg/y)	0.0 ± 10.2	0.0 ± 10.5	0.0 ± 9.8	1.00
Calf muscle area at 66% tibia (mm^2)	6 411.1 ± 1 254.2	5 765.1 ± 946.9	7 120.8 ± 1 164.6	<.0001
Calf fat area at 66% tibial length level (mm^2)	1 861.3 ± 1 134.6	2 528.2 ± 1 119.7	1 128.5 ± 547.4	<.0001
Plasma GDF-15 (RFU)	1 943.5 (1 558.1–2 463.3)	1 840.5 (1 479.5–2 387.2)	2 016.4 (1 625.6–2 569.8)	<.0001
Serum 25 (OH)D (nmol/L)	44.2 (30.0–68.3)	36.2 (24.7–52.5)	58.0 (39.2–76.9)	<.0001
Serum calcium (mg/dL)	9.5 ± 0.4	9.5 ± 0.5	9.4 ± 0.4	.01
Serum parathyroid hormone (pg/mL)	21.6 (15.3–30.2)	23.6 (15.8–34.2)	19.8 (14.6–26.7)	.21
Total bone density (mg/cm^3)	257.6 ± 47.5	238.2 ± 44.2	278.9 ± 41.6	<.0001
Cortical bone density (mg/cm^3)	1 101.9 ± 77.8	1 083.0 ± 82.0	1 122.8 ± 67.0	.73
Trabecular bone density (mg/cm^3)	207.5 ± 54.2	198.9 ± 58.8	216.9 ± 46.9	.0001
Medullary + trabecular bone density (mg/cm^3)	476.9 ± 45.3	477.0 ± 42.8	476.8 ± 47.9	.73
Total bone area (mm^2)	385.5 ± 69.9	337.7 ± 43.8	438.2 ± 53.5	<.0001
Cortical bone area (mm^2)	301.9 ± 71.1	251.6 ± 47.4	357.1 ± 47.9	<.0001
Medullary bone area (mm^2)	84.3 ± 31.3	86.9 ± 35.9	81.4 ± 25.2	.06
Minimum moment of inertia (g/cm)	1 065.5 ± 361.5	820.4 ± 200.3	1 334.7 ± 302.7	<.0001

Notes: GDF-15 = growth and differentiation factor 15; RFU = relative fluorescence units; *SD* = standard deviation. Values are presented as mean ± *SD* or median (interquartile). *Except for age, p values were obtained from an age-adjusted linear regression model for continuous variables and logistic regression model for categorical variables.

Table 2. Participant Characteristics Stratified by Sex-Specific Median GDF-15 at Baseline

	Women			Men		
	High GDF-15 (n = 156)	Low GDF-15 (n = 156)	p Value	High GDF-15 (n = 142)	Low GDF-15 (n = 142)	pValue
Age (y)	76.1 ± 6.4	71.2 ± 4.9	**<.0001**	74.8 ± 6.8	70.7 ± 4.2	**<.0001**
Follow-up time (y)	6.2 ± 3.4	7.6 ± 2.4	.08	6.4 ± 3.2	7.8 ± 2.4	.18
Education (≥6 y, %)	15.38	16.67	.94	33.1	50	.10
Current smoker (%)	9.62	5.77	.07	29.58	16.9	**.002**
Inactive (%)	25	12.18	.23	12.68	3.52	**.02**
Diabetes (%)	16.03	6.41	**.01**	16.9	8.45	**.01**
Body mass index (kg/m²)	28.2 ± 4.9	27.9 ± 4.3	.11	26.5 ± 3.6	27.4 ± 2.9	.37
Annual rate of change in body weight (kg/y)	0.7 ± 11.1	−0.7 ± 9.8	.37	−0.5 ± 10.5	0.5 ± 9.0	.37
Calf muscle area at 66% tibia (mm²)	5 632.5 ± 934.0	5 897.8 ± 944.1	.18	6 952.4 ± 1 192.9	7 289.2 ± 1 114.6	.99
Calf fat area at 66% tibia (mm²)	2 506.9 ± 1 163.2	2 549.5 ± 1 077.8	.33	1 088.9 ± 567.0	1 168.2 ± 526.0	.42
Serum 25 (OH)D (nmol/L)	32.3 (22.0–49.8)	39.6 (28.3–61.9)	.10	49.9 (35.4–73.9)	59.7 (43.4–77.4)	.25
Serum calcium (mg/dL)	9.5 ± 0.5	9.5 ± 0.5	.98	9.4 ± 0.4	9.4 ± 0.3	.70
Serum parathyroid hormone (pg/mL)	24.7 (16.3–36.9)	22.3 (14.9–29.8)	.11	21.4 (14.5–28.3)	19.0 (14.7–24.2)	.99
Total bone density (mg/cm³)	237.9 ± 44.9	238.4 ± 43.7	.14	274.0 ± 41.1	283.7 ± 41.6	.12
Cortical bone density (mg/cm³)	1 079.1 ± 78.5	1 086.8 ± 85.4	.38	1 120.4 ± 70.1	1 125.1 ± 64.0	.10
Trabecular bone density (mg/cm³)	202.3 ± 61.6	195.5 ± 55.9	.06	210.8 ± 46.5	222.9 ± 46.7	.10
Medullary + trabecular bone density (mg/cm³)	469.4 ± 41.6	484.6 ± 42.8	.07	474.8 ± 50.0	478.8 ± 45.9	.88
Total bone area (mm²)	337.9 ± 44.4	337.6 ± 43.4	.37	438.3 ± 52.4	438.1 ± 54.7	.46
Cortical bone area (mm²)	248.0 ± 48.6	255.3 ± 46.1	.31	355.7 ± 49.4	9.4 ± 0.3	.62
Medullary bone area (mm²)	91.3 ± 37.0	82.6 ± 34.3	.96	83.8 ± 27.3	78.9 ± 22.7	.12
Minimum moment of inertia (g/cm)	818.4 ± 209.8	822.3 ± 191.1	.42	1 353.2 ± 317.4	1 316.2 ± 287.2	.13

Notes: GDF-15 = growth and differentiation factor 15; RFU = relative fluorescence units; *SD* = standard deviation. Values are presented as mean ± *SD* or median (interquartile). *Except for age, p values were obtained from an age-adjusted linear regression model for continuous variables and logistic regression model for categorical variables. Median value of GDF-15 was 2 016.4 RFU for men, 1 840.5 RFU for women, respectively. Bold numbers represent statistically significant difference between high GDF-15 and low GDF-15 individuals.

Table 3. Association Between Baseline Plasma GDF-15 and Rate of Change in Bone Parameters

	Women				Men			
	β	*SE*	p Value	FDR	β	*SE*	p Value	FDR
Total bone density (mg/cm³)	−3.38	1.69	.046	0.09	−1.92	0.74	.01	0.08
Cortical bone density (mg/cm³)	−7.37	2.00	.0002	**0.001**	−0.25	2.29	.91	0.94
Trabecular bone density (mg/cm³)	−3.83	1.83	.04	0.09	0.23	1.01	.82	0.94
Medullary + trabecular bone density (mg/cm³)	−5.32	4.62	.25	0.33	−4.52	4.94	.36	0.58
Total bone area (mm²)	−0.42	1.20	.73	0.73	−2.75	1.54	.08	0.16
Cortical bone area (mm²)	1.00	1.18	.4	0.46	−3.49	1.73	.04	0.11
Medullary bone area (mm²)	−1.46	1.07	.17	0.27	−0.12	1.66	.94	0.94
Minimum moment of inertia (g/cm)	0.37	0.37	.0003	**0.001**	−14.55	7.17	.04	0.11

Notes: FDR = false discovery rate; GDF-15 = growth and differentiation factor 15; PTH = intact parathyroid hormone; *SE* = standard error. β (*SE*) represents the interaction term between $\log_{10}$ (plasma GDF-15) and time (years). Linear mixed-effect models were adjusted for age centered at 70 years, age centered at 70 years × age centered at 70 years, body mass index, slope of body weight, $\log_{10}$ (plasma GDF-15), education level, current smoking status, physical activity (inactive), diabetes mellitus, calf muscle mass, calf fat mass, $\log_{10}$ (25 (OH)D), $\log_{10}$ (PTH), and calcium. Bold numbers represent statistically significant association between baseline plasma GDF-15 and rate of change in bone parameter by false discovery rate.

Discussion

This is among the first studies to identify an association between GDF-15 and an accelerated rate of loss of bone integrity assessed using multiple parameters in community-dwelling adults aged ≥65 years. Our major findings are (a) consistent with the literature, women had accelerated declines in several bone parameters compared to men and (b) higher circulating levels of GDF-15 were associated with significantly accelerated declines in bone densities, especially cortical bone density, and in the mMOI in women but not in men. These findings are generally consistent with prior work in this cohort that observed larger declines in bone parameters in women than in men over a 6-year follow-up (22,32). In postmenopausal women, trabecular bone declines rapidly over 3–5 years, followed by a slow decline in both cortical and trabecular bone over several decades. In men, this slow loss of bone mass is seen without the initial rapid loss of bone mass (33–35). Differences in long bone geometry between men and women are well-established (36). In men, the increase in cortical thickness is mainly due to periosteal apposition, which results in a thicker cortex, while in women, its increase is mainly due to increasing endosteal deposition. After menopause, cortical bone becomes thinner due to increased endocortical resorption and decreased periosteal apposition. Considering the sample mean age (70 years in women), bone densities in each bone region moderately decreased in both sexes, but bone densities in women showed a steeper decrease than those in men.

It is likely that differences in sex hormonal characteristics between men and women may explain the differential effect of GDF-15 on change in bone density loss in men and women. Age-related decreases in sex hormones (androgens and estrogens) are primary mechanisms for bone loss in women. Estrogen plays a protective role in bone remodeling in undifferentiated osteoblasts, differentiated osteoblasts, and even differentiated osteocytes (37–39). After menopause, lower estrogen levels lead to a rapid increase in bone turnover, subsequently resulting in a rapid decrease in bone mass (40). In addition to its direct effect on bone tissues, estrogen indirectly regulates bone metabolism via the immune system (41). The effects of age-related decreases in sex hormones on bone density are not limited to women. Indeed, epidemiological studies demonstrated that osteopenia progressed over the years and the numbers of osteoporotic and fragility fractures increased over the years, particularly in men ≥75 years (42). Furthermore research is required to examine the association between sex hormones, circulating levels of GDF-15, and bone tissue in both sexes, especially in later life.

Cellular senescence is another major mechanism of bone fragility. The secretion by senescent cells of the SASP, which is comprised of inflammatory chemokines, cytokines, and extracellular matrix remodeling enzymes, has been implicated in bone fragility (43). Oxidative stress is an upstream regulator of cellular senescence and SASP, while estrogen has antioxidative effects (41,44). Taken together, the 2 major mechanisms of bone fragility are likely to be interrelated. Coupled with lower estrogen levels after menopause, higher circulating level of GDF-15, which may represent a state of accumulated senescent cells, may contribute to bone fragility. Furthermore studies are needed to examine underlying mechanisms of these sex differences.

The biological mechanisms by which GDF-15 causes accelerated bone loss are not fully understood. With aging, the oxygen supply to tissues decreases. Hinoi et al. showed that osteocytes under hypoxic conditions regulate osteoclast differentiation via GDF-15 (19). GDF-15, whose expression is upregulated in hypoxic osteocytes, increases tartrate-resistant acid phosphatase-positive multinucleated cells, a marker of osteoclast differentiation, in a dose-dependent manner (19). In addition, GDF-15 alone does not induce phosphorylation of p65 and its inhibitory protein IκB, but promotes phosphorylation of p65 in the presence of receptor activator of nuclear factor-κB ligand (RANKL), suggesting that GDF-15 promotes RANKL-induced osteoclast differentiation through NF-κB signaling (19). In preclinical studies, anti-GDF-15 antibody inhibited bone loss and osteoclast activation in hypoxic bone. This result further supports our finding that circulating GDF-15 is linked to accelerating bone loss. On the other hand, the role of GDF-15 in osteoblast differentiation remains unclear; Westhrin et al. showed that GDF-15 inhibits alkaline phosphatase activity, an early marker of osteoblast differentiation, in a dose-dependent manner, in vitro (human bone marrow-derived mesenchymal stem cells) (18). Wakchoure et al. found that GDF-15 was associated with TLR9-mediated osteoclast and osteoblast differentiation under tumor growth conditions, but not under vehicle control conditions (45).

In our sample, the mMOI remained stable in both men and women throughout the 9-year follow-up period. Our previous cross-sectional studies have shown that the mMOI is lower with increasing age in women but not in men (22,23). There may be discrepancies between these previous studies and the present study, depending on whether interindividual variation was taken into account. Considering the lack of change in the mMOI, the result of a significant association between plasma GDF-15 and the rate of change in the mMOI was somewhat surprising. In women with higher GDF-15, the mMOI was lower at baseline and decreased more rapidly over time relative to women with lower GDF-15. Although it is still unclear to what extent the decrease in mMOI contributes to bone fragility and fracture, the present results suggest that high plasma GDF-15 in women is a biomarker of bone fragility in women, and perhaps also a risk factor for fractures.

Our study has several strengths, including a population-based sample, standardized data collection, and over 9 years of follow-up with low attrition other than mortality. Some limitations include a lack of ethnic diversity, as all study participants were Italian, and enrollment was targeted at older age participants who may have experienced substantial bone loss prior to enrollment. Future studies are needed to examine the association between GDF-15 and osteopenia in younger subjects when trabecular bone starts to decline. Second, because the present study used a targeted approach to examine the association between a single biomarker and bone parameters, we could not identify an underlying biomarker network that, together with GDF-15, may play a role in the process of bone loss (46). Furthermore studies are needed to explore clusters of multiple blood biomarkers that may predict bone fragility. Third, it would have been interesting to examine the association between the rate of change in bone parameters and future bone-related events such as osteoporosis onset and fracture. However, the number of participants who had bone-parameters for 9-year follow-ups and then experienced fractures after the follow-up visit was very limited. Last, because we did not measure DXA at the femoral neck or spine, we did not evaluate the prevalence rate of osteoporosis, and our samples might therefore have included osteoporotic individuals at baseline.

This study demonstrated that the plasma GDF-15 level is a promising biomarker to predict accelerated declines in bone parameters, especially in women. Furthermore investigation is needed to

examine the roles of GDF-15 in the biological pathways leading to bone fragility.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None declared.

Data Availability

Proteomic data generated from this study are available upon request through submission of proposals at the InCHIANTI study website http://inchiantistudy.net/wp/.

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