Postprint on Primary Osteoporosis and the Muscle-Bone-Fat Relationship

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Date: 2022-08-12T00:00:00+00:00

Abstract

Background: Osteoporosis, as a chronic age-related disease, is characterized by a slow and insidious onset, and has long been referred to as the "silent killer" in academic circles. Currently, research on the relationship between the "muscle-bone-fat" axis and the pathogenesis of osteoporosis remains limited. Objective: To measure subjects' T-scores, fat mass, muscle mass, and bone mineral content-related parameters, record general information, calculate BMI and fat percentage, and explore the relationship between osteoporosis and the muscle-bone-fat axis. Methods: A total of 108 subjects were enrolled. General information was recorded. Dual-energy X-ray absorptiometry (DXA) was used to measure subjects' T-scores, fat mass, muscle mass, and bone mineral content. Enzyme-linked immunosorbent assay (ELISA) was employed to detect serum bone formation-related markers including calcium, bone morphogenetic protein-2 (BMP2), and osteoprotegerin (OPG). Subjects were grouped according to age ranges, and one-way analysis of variance (ANOVA) was used to compare height, weight, BMI, T-score, fat mass, muscle mass, fat percentage, and bone mineral content among different age groups. Based on T-scores, subjects were divided into normal, osteopenia, and osteoporosis groups, and comparisons were made among groups for fat mass, muscle mass, fat percentage, bone mineral content, and bone formation-related serum markers. Results: Statistically significant differences in T-scores were observed among different age groups ($P<0.05$), with highly significant differences between the $ $69 years group and the 49-58 years group ($P<0.01$). Significant differences in fat mass, muscle mass, and bone mineral content were found among different bone density groups ($P<0.05$), with highly significant differences in bone mineral content ($P<0.01$). Compared with the normal group, bone mineral content in the osteopenia and osteoporosis groups differed highly significantly ($P<0.01$), while fat content in the osteoporosis group differed significantly ($P<0.05$). Expression levels of OPG, BMP2, and BCL2 differed significantly among bone density groups ($P<0.05$). Compared

with the normal group, BMP2 expression in the osteopenia and osteoporosis groups differed significantly (P<0.05), and OPG expression in the osteoporosis group differed significantly (P<0.05). Conclusion: The dynamic balance of the "muscle-bone-fat" axis is closely associated with the pathogenesis of osteoporosis. With advancing age, human bone density exhibits a declining trend; bone density represents a crucial factor influencing fat mass, muscle mass, and bone mineral content. Reduced serum levels of BMP2, OPG, and Bcl2 may be associated with the inability of these proteins to effectively exert their anti-apoptotic and pro-osteogenic functions, leading to decreased osteoblast activity and diminished bone formation.

Full Text

Preamble

Analyzing the Expression Differences of Muscular-Bone-Lipid Related Indexes in Different Age Groups and Bone Density Groups

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Funding: National Natural Science Foundation of China (No. 81973886); Guangdong Province Graduate Education Innovation Program Project (2022XSLT013); Guangzhou University of Chinese Medicine "Double First-Class" and High-Level University Discipline Collaborative Innovation Team Key Project (2021XK21).

Abstract

Background: Osteoporosis, as a chronic age-related disease, has a slow and insidious onset process and is known as a "silent killer" in the academic community. Currently, there are few studies investigating the relationship between the "muscle-bone-fat" axis and osteoporosis pathogenesis. **Objective:** To measure participants' T-scores, fat mass, muscle mass, and bone mineral content-related indicators, record general information, calculate BMI and fat percentage, and explore the relationship between osteoporosis and the muscle-bone-fat axis. **Methods:** A total of 108 participants were enrolled. General data were recorded, and dual-energy X-ray absorptiometry (DXA) was used to detect T-scores, fat mass, muscle mass, and bone mineral content. ELISA was used to

detect bone formation-related serum indicators including calcium, bone morphogenetic protein-2 (BMP2), and osteoprotegerin (OPG). Participants were grouped by age, and one-way ANOVA was used to compare height, weight, BMI, T-scores, fat mass, muscle mass, fat percentage, and bone mineral content across different age groups. Based on T-scores, participants were divided into normal, osteopenia, and osteoporosis groups to compare fat mass, muscle mass, fat percentage, bone mineral content, and bone formation-related serum indicators. **Results:** T-scores differed significantly among age groups ($P < 0.05$), with particularly significant differences between the $ $69 years group and the 49–58 years group ($P < 0.01$). Fat mass, muscle mass, and bone mineral content differed significantly among bone density groups ($P < 0.05$), with bone mineral content showing highly significant differences ($P < 0.01$). Compared with the normal group, bone mineral content in both the osteopenia and osteoporosis groups showed highly significant differences ($P < 0.01$), while fat content in the osteoporosis group showed significant differences ($P < 0.05$). OPG, BMP2, and BCL2 expression differed significantly among bone density groups ($P < 0.05$). Compared with the normal group, BMP2 expression in the osteopenia and osteoporosis groups differed significantly ($P < 0.05$), and OPG expression in the osteoporosis group differed significantly ($P < 0.05$). **Conclusion:** The dynamic balance among muscle-bone-fat is closely related to osteoporosis pathogenesis. Bone mineral density declines with age and is an important factor affecting fat mass, muscle mass, and bone mineral content. Decreased serum BMP2, OPG, and Bcl2 levels may be associated with impaired anti-apoptotic and pro-osteogenic functions of these proteins, leading to reduced osteoblast activity and weakened bone formation.

Keywords: Osteoporosis; Muscular tissue; Skeletal system; Adipose tissue

Introduction

Osteoporosis (OP) is a common skeletal disease characterized by reduced bone mass, deterioration of bone microarchitecture, increased bone fragility, and susceptibility to fracture [1]. The current gold standard for diagnosing osteoporosis is dual-energy X-ray absorptiometry (DXA) for measuring bone mineral density, which offers convenient operation with high accuracy and precision [2]. Additionally, DXA serves as the standard method for body composition measurement, enabling acquisition of whole-body and regional measurements of muscle, fat, bone mineral content, and their ratios, thereby providing clinical data support for exploring the relationship between osteoporosis and the muscle-bone-fat axis.

1.1 Diagnostic Criteria

The diagnostic criteria for osteoporosis referenced the WHO-recommended standards for primary osteoporosis from 2006, based on bone mineral density measurements. Using DXA to measure anteroposterior lumbar spine (L1–L4) bone

mineral density (BMD): BMD > M-1SD is normal; BMD between M-1SD and M-2.5SD indicates bone loss; BMD < M-2.5SD indicates osteoporosis; and BMD < M-2.5SD with one or more fractures indicates severe osteoporosis.

1.2 Inclusion Criteria

1. Women aged $ $49 years who were postmenopausal for >1 year, and men aged $ $60 years

2. No prior systematic osteoporosis treatment, with obvious clinical symptoms

3. No severe heart, lung, liver, or kidney dysfunction or bone metabolic diseases

4. Understanding of the study and voluntary participation

1.3 Exclusion Criteria

1. Patients with secondary osteoporosis (endocrine diseases such as hyperthyroidism and diabetes; autoimmune diseases such as rheumatoid arthritis and ankylosing spondylitis; hematological diseases such as multiple myeloma; digestive and renal diseases affecting calcium and vitamin D absorption and metabolism; neuromuscular diseases; and osteoporosis caused by other conditions like COPD)

2. Patients who had taken medications affecting bone metabolism within 6 months

3. Patients with other comorbidities

4. Patients who did not consent to or cooperate with the study

1.4 Participants

From July 19, 2019, to January 5, 2021, our hospital recruited 133 cases, all permanent residents of Guangzhou. A total of 108 participants met the inclusion criteria, including 97 females (89.81%) and 11 males (10.09%), aged 49–88 years with a mean age of 62.87 ± 7.06 years. This study was approved by the Medical Ethics Committee of the Third Affiliated Hospital of Guangzhou University of Chinese Medicine (approval number: 2020034).

1.5 Bone Mineral Density Detection and Body Composition Measurement

A dual-energy X-ray bone densitometer was used to detect bone mineral density in the anteroposterior lumbar spine (L1–L4) and left proximal femur, including

the left femoral neck, Ward' s triangle, greater trochanter, and total femur. Diagnostic criteria referenced the 2017 Chinese Guidelines for the Diagnosis and Treatment of Primary Osteoporosis [2]: DXA-measured peak bone mass in the central skeleton (M ± SD) served as the normal reference value. >M-1SD was normal, ≤M-1SD to -2SD indicated osteopenia, <M-2SD indicated osteoporosis, and <M-2SD with one or more fractures indicated severe osteoporosis. The bone densitometer was used to measure whole-body muscle mass and fat mass, and fat percentage was calculated.

1.6 Bone Formation-Related Indicator Detection

Fasting peripheral blood (4 mL) was collected from each participant after fasting for >10 hours, with venous blood drawn from the elbow between 9:00–10:00 AM. ELISA was used to detect serum calcium, osteoprotegerin (OPG), bone morphogenetic protein-2 (BMP2), and B-cell lymphoma-2 gene (BCL2).

1.7 Statistical Methods

SPSS 25.0 statistical software was used. Measurement data were expressed as mean ± standard deviation. One-way ANOVA was used for multi-group comparisons, and Bonferroni test was used for multiple comparisons between groups. $P > 0.05$ indicated no statistically significant difference, $P < 0.05$ indicated statistically significant difference, and $P < 0.01$ indicated highly statistically significant difference.

Results

2.1 Comparison of General Participant Data

There were no statistically significant differences in height, weight, or BMI among different age groups ($P > 0.05$) . However, T-scores differed significantly among age groups ($P < 0.05$), with significant differences between the 59–68 years group and the 49–58 years group ($P < 0.05$), and highly significant differences in the $ $69 years group compared with the 49–58 years group ($P < 0.01$).

Table 1 Comparison of Height, Weight, BMI, and T-Scores Between Different Age Groups (Mean ± SD)

Age Group	Height (m)	Weight (kg)	BMI (kg/m^2)	T-Score
49–58	1.58 ± 0.06	54.46 ± 6.83	21.63 ± 2.70	-1.65 ± 1.26
59–68	1.57 ± 0.05	55.21 ± 7.59	22.33 ± 3.08	-2.34 ± 1.42*
$ $69	1.56 ± 0.06	56.08 ± 8.13	22.96 ± 3.23	-2.73 ± 1.31**

T-score = (Measured value - Peak bone density of healthy young adults of same race and sex) / Standard deviation of peak bone density of healthy young adults of same race and sex

Note: indicates statistically significant difference compared with 49-58 years group; ** indicates highly statistically significant difference compared with 49-58 years group.*

2.2 Comparison of Fat Mass, Muscle Mass, Fat Percentage, and Bone Mineral Content

There were no statistically significant differences in fat mass, muscle mass, fat percentage, or bone mineral content among different age groups ($P > 0.05$) . Based on participants' T-scores, they were divided into normal, osteopenia, and osteoporosis groups. Analysis results for fat mass, muscle mass, fat percentage, and bone mineral content are shown in Table 3. There were no statistically significant differences in fat percentage among groups ($P > 0.05$), but significant differences were found in fat mass, muscle mass, and bone mineral content ($P < 0.05$), with bone mineral content showing highly significant differences ($P < 0.01$). Compared with the normal group, the osteoporosis group showed significant differences in fat content ($P < 0.05$), and both osteopenia and osteoporosis groups showed highly significant differences in bone mineral content ($P < 0.01$). Compared with the osteopenia group, the osteoporosis group showed significant differences in muscle mass ($P < 0.05$) and highly significant differences in bone mineral content ($P < 0.01$).

Table 2 Comparison of Fat Mass, Muscle Mass, Fat Percentage, and Bone Mineral Content in Different Age Groups (Mean ± SD)

Age Group	n	Fat Mass (g)	Muscle Mass (g)	Fat Percentage (%)	Bone Mineral Content (g)
49-58	31	18664.01 ± 4133.41	34070.78 ± 3356.28	33.49 ± 4.77	1715.36 ± 233.90
59-68	57	18313.35 ± 4159.07	35110.87 ± 5178.73	33.14 ± 4.76	1613.11 ± 312.45
$ $69	20	18404.96 ± 4050.80	35495.88 ± 6179.41	33.23 ± 6.13	1536.87 ± 300.01

Table 3 Comparison of Fat Mass, Muscle Mass, Fat Percentage, and Bone Mineral Content Among Normal, Osteopenia, and Osteoporosis Groups (Mean ± SD)

Group	n	Fat Mass (g)	Muscle Mass (g)	Fat Percentage (%)	Bone Mineral Content (g)
Normal	20	19765.63 ± 5214.64	35780.18 ± 5093.34	33.25 ± 5.95	2022.73 ± 223.09
Osteopenia	38	19086.44 ± 4154.20	36202.62 ± 4881.37	33.37 ± 5.65	1712.66 ± 168.10**
Osteoporosis	50	17398.95 ± 3304.62*	33522.57 ± 4622.30#	33.18 ± 4.08	1406.50 ± 169.09**##

Note: indicates statistically significant difference compared with normal group; ** indicates highly statistically significant difference compared with normal group; # indicates statistically significant difference compared with osteopenia group; ## indicates highly statistically significant difference compared with osteopenia group.*

2.3 Comparison of Bone Formation-Related Indicators

Based on participants' T-scores, they were divided into normal, osteopenia, and osteoporosis groups. Bone formation-related indicator detection results are shown in Table 4. There were no statistically significant differences in Ca^{2+} expression among groups ($P > 0.05$). However, OPG, BMP2, and BCL2 expression differed significantly ($P < 0.05$). Compared with the normal group, the osteoporosis group showed significant differences in OPG expression ($P < 0.05$) and highly significant differences in BMP2 expression ($P < 0.01$), while the osteopenia group showed significant differences in BMP2 expression ($P < 0.05$). Compared with the osteopenia group, the osteoporosis group showed significant differences in OPG and BCL2 expression ($P < 0.05$).

Table 4 Comparison of Bone Formation-Related Indicators Among Normal, Osteopenia, and Osteoporosis Groups (Mean ± SD)

Group	n	Ca (mmol/L)	OPG (pg/mL)	BMP2 (ng/L)	BCL2 (g/L)
Normal	20	1.24 ± 0.19	3665.67 ± 3139.33	653.47 ± 184.44	161.88 ± 158.01
Osteopenia	38	1.28 ± 0.19	3352.63 ± 3044.51	458.87 ± 262.95*	173.93 ± 154.43
Osteoporosis	50	1.23 ± 0.18	2062.60 ± 1474.09*#	441.45 ± 322.46**	106.67 ± 60.08#

Note: indicates statistically significant difference compared with normal group; ** indicates highly statistically significant difference compared with normal

group; # indicates statistically significant difference compared with osteopenia group.*

Discussion

Osteoporosis is a progressive, age-related metabolic bone disease characterized by generalized bone loss, accompanied by deterioration of bone microarchitecture, leading to decreased bone density and increased fracture risk [3,4]. DXA is the preferred method for diagnosing osteoporosis, and bone mineral density is an important predictor of fracture risk [5], enabling both fracture risk prediction and patient monitoring. The T-score represents the number of standard deviations from the peak bone density of healthy young adults of the same race and sex. A T-score of 0 means the subject' s bone density equals that of healthy young adults, while increasingly negative T-scores indicate lower bone density and higher fracture risk [6]. According to WHO-recommended diagnostic criteria, BMD values within 1 standard deviation below the peak bone mass of healthy adults of the same sex and race are normal; reductions of 1–2.5 standard deviations indicate low bone mass; and reductions of $ $2.5 standard deviations indicate osteoporosis [7]. Population surveys in the UK and US have confirmed that bone density declines with age in the general population, a finding validated in studies of osteoporotic fracture patients, while body mass index (BMI) is a risk factor for hip fracture [8,9]. Our results showed no statistically significant differences in height, weight, or BMI among different age groups ($P > 0.05$), but T-scores differed significantly ($P < 0.05$), with significant differences in the 59–68 years group and highly significant differences in the $ $69 years group compared with the 49–58 years group ($P < 0.01$). These findings demonstrate that bone density declines with age in our study population, providing clinical evidence for strengthening osteoporosis prevention and fracture prevention in the elderly.

Aging is accompanied by numerous physiological changes, with particularly evident phenotypic changes in muscle, bone, and fat. Human muscle mass peaks between ages 30–40 and then gradually declines [11], with surveys showing that muscle mass decreases by 0.45% annually in men and 0.37% in women [12]. Age-related muscle strength decline is an important predictor of functional disability, falls, fractures, and mortality in older adults [13,14]. Sarcopenia is an age-related disease characterized by decreased muscle mass and function, with increased fall risk elevating fracture risk. In normal-weight adults, skeletal muscle accounts for approximately 40% of total body weight, making it one of the largest organs [15]. Moreover, skeletal muscle is considered an endocrine organ that produces and releases various factors such as osteogenic growth factors, interleukin-6 (IL-6), insulin-like growth factor 1 (IGF-1), and fibroblast growth factor 2 (FGF-2), exerting paracrine, autocrine, and endocrine effects that influence bone health [16–18]. Obesity incidence is rising annually, predisposing individuals to various musculoskeletal complications and metabolic disorders [19]. Conversely, fat accumulation increases with age, in contrast to the de-

cline in muscle and bone tissue, and may plateau or decline in very old age [20]. Additionally, with aging, fat is redistributed to intra-abdominal regions and infiltrates muscle and bone, all contributing to decreased overall strength and function and increased fall and fracture risk [11]. Our study found no statistically significant differences in fat mass, muscle mass, fat percentage, or bone mineral content among different age groups ($P > 0.05$). However, when participants were divided into normal, osteopenia, and osteoporosis groups based on T-scores, significant differences emerged in fat mass, muscle mass, and bone mineral content ($P < 0.05$), with highly significant differences in bone mineral content ($P < 0.01$). Compared with the normal group, the osteoporosis group showed significant differences in fat content ($P < 0.05$), and both osteopenia and osteoporosis groups showed highly significant differences in bone mineral content ($P < 0.01$). Compared with the osteopenia group, the osteoporosis group showed significant differences in muscle mass ($P < 0.05$) and highly significant differences in bone mineral content ($P < 0.01$). These findings suggest that, compared with age, bone density is more likely to be an important factor affecting fat mass, muscle mass, and bone mineral content, which has important clinical implications for diagnosing and treating osteopenia or osteoporosis. We speculate that improving patients' bone density may ameliorate functional impairment and falls caused by reduced muscle mass, though the specific mechanisms require further investigation.

Bone remodeling is regulated by osteoclasts and osteoblasts, with various cytokines and hormones modulating activation, resorption, reversal, formation, and termination phases [21]. RANKL and OPG are members of the TNF superfamily that are crucial for regulating bone resorption. RANKL activates osteoclast formation, activation, and survival through its receptor RANK, while OPG inhibits osteoclastogenesis and bone loss by binding to RANKL, thereby preventing RANK-RANKL binding [22]. Based on T-scores, we divided participants into normal, osteopenia, and osteoporosis groups for bone formation-related indicator detection. Results showed significant differences in OPG expression among groups ($P < 0.05$), with OPG expression decreasing as T-scores declined. Compared with the normal group, the osteoporosis group showed significant differences in OPG expression ($P < 0.05$), and compared with the osteopenia group, the osteoporosis group also showed significant differences ($P < 0.05$), indicating that bone remodeling is destabilized in osteopenia and osteoporosis populations, with accelerated bone resorption and bone loss.

Bone formation is closely related to osteoblast activity [23]. In healthy individuals, a balance exists between osteoclast and osteoblast activity. In osteoporosis patients, this relationship becomes imbalanced, with increased osteoclast activity and decreased osteoblast activity [24], leading to increased skeletal porosity and fracture risk.

BMP2 is a potent growth factor that induces both osteoblast and osteoclast activity [25]. BMP2 binds to two receptors responsible for initiating canonical and non-canonical signaling cascades: type I receptor (BMPRIa) and type II recep-

tor (BMPRII). BMP2-induced signaling is initiated when the protein recruits and binds to these receptors, forming a complex where the type II receptor phosphorylates the type I receptor [26], subsequently activating both SMAD-dependent and independent pathways [27]. While most research has focused on drug effects on BMP2 protein in rats, our study examined human serum BMP2 levels. We found significant differences in BMP2 expression among groups ($P < 0.05$), with significant differences in the osteopenia group ($P < 0.05$) and highly significant differences in the osteoporosis group ($P < 0.01$) compared with the normal group. These findings suggest that serum BMP2 levels are lower in osteopenia and osteoporosis populations, possibly related to reduced osteoblast activity and weakened bone formation. Investigating BMP2 is important for elucidating the activation mechanisms of osteoblasts and osteoclasts and may provide clues for future osteoporosis treatment.

Bcl2 is an anti-apoptotic protein that primarily inhibits apoptosis by regulating mitochondrial permeability changes, including suppressing oxidant-induced apoptosis, inhibiting intracellular calcium transmembrane movement, and forming ion channels to inhibit apoptosis [28]. Our research team previously found differential expression of Bcl2-related genes in osteoporosis patients [29–31]. Based on this, we constructed Bcl2 overexpression and silencing adenoviruses and transfected osteoblasts and rats, finding that silencing Bcl2 expression inhibited MG63 cell proliferation and osteogenic differentiation capacity, while Bcl2 recombinant adenovirus vector transfection enhanced cell viability and mineralization capacity, promoting bone formation. Overexpressing Bcl2 can promote BMP2 and OPG protein expression, though whether other apoptotic proteins participate and whether cascade reactions occur requires further investigation. Our study found significant differences in Bcl2 expression among groups ($P < 0.05$), with significant differences between the osteoporosis and osteopenia groups ($P < 0.05$). Compared with the normal group, the osteoporosis group had lower Bcl2 content, consistent with our previous research. We hypothesize that in osteoporosis populations, Bcl2 protein cannot effectively exert anti-apoptotic and pro-osteogenic effects, further inducing osteoporosis.

Conclusion

Osteoporosis, as a chronic age-related disease with a slow and insidious onset, is known as a "silent killer." The dynamic balance among muscle-bone-fat is closely related to its pathogenesis, with these three components interconnected, interfering with, and influencing each other. When any one component becomes pathological, the mechanical balance in this relationship is disrupted, leading to disease onset. Our findings demonstrate that bone density declines with age and is an important factor affecting fat mass, muscle mass, and bone mineral content. Decreased serum BMP2, OPG, and Bcl2 levels may be associated with impaired anti-apoptotic and pro-osteogenic functions, leading to reduced osteoblast activity and weakened bone formation. A limitation of this study is the lack of in-depth correlation analysis between primary osteoporosis and

the muscle-bone-fat axis. Currently, few studies have examined the connections among these three components, and our research aims to explore their mutual influences in osteoporosis pathogenesis, providing a clinical basis for further experimental research.

Author Contributions

Lin Yanping: conceptualization and design, manuscript writing; Huang Jiachun, Yuan Jiayao, Lin Xiancan: data collection and organization; Guo Haiwei: study implementation; Wan Lei: feasibility analysis; Huang Hongxing: quality control and manuscript review, overall responsibility, supervision.

Conflict of Interest

The authors declare no conflict of interest.

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Note: Figure translations are in progress. See original paper for figures.