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Community series - reducing the burden of age-related disease in relation to osteoporosis, sarcopenia and osteosarcopenia, volume II

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Community series - reducing the burden of age-related disease in relation to osteoporosis, sarcopenia and osteosarcopenia, volume II

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Editorial: Community series - reducing the burden of age-related disease in relation to osteoporosis, sarcopenia and osteosarcopenia, volume II

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Editorial on the Research Topic
Community series - reducing the burden of age-related disease in relation to osteoporosis, sarcopenia and osteosarcopenia, volume II

Considering the accelerated aging rate, doubled to 1.5 billion by 2050, the cost and burden of related health problems are increasing. One of the more prevalent health problems among the elderly population is frailty that finally can result in sarcopenia and osteoporosis which may increase the fracture rate. This issue therefore aimed to report novel advancements in these two areas, focusing on new preventive measures and treatment options. The effect of certain co-morbidities on these two conditions were also highlighted, suggesting that preventing osteoporosis/sarcopenia can also reduce other co-morbidities among the elderly, improving their quality of life.

Three of the articles published in this issue were focused on sarcopenia (Merchant et al., Diniz de Salles et al., Hou et al.). The beneficial effects of nutrition in the pre-frailty phase on the prevention or reduction of frailty and disability risk in older adults is well known (1). Leucine, and leucine-enriched protein supplements in the range of the recommended dietary allowance (RDA) for protein in older adults (0.8 g/kg per day), together with resistance exercise, have been shown to be beneficial for muscle mass, physical function, and systemic inflammation. However, the effect of an additional dose of protein on RDA when combined with exercise in the elderly population is not yet known. In a non-randomized trial study,

Merchant et al. assessed the short-term effect (3 months) of a diet enriched with an additional protein and leucine supplementation with/without exercise on the physical function, muscle mass, and systemic inflammation in prefrail older adults who had received RDA protein. They found a significant improvement in body cell mass, and systemic inflammation in both groups; short physical performance battery (SPPB) test, gait speed, 5× sit-to-stand (STS), and muscle mass, however, improved significantly only in the nutrition+ exercise group. Since these effects were not sustained after a 3-month follow-up, their findings should be confirmed in future randomized trials with a larger number of at-risk elderly.

Elderly patients are at increased risk of postoperative complications (2), which require rapid recognition and treatment. Otherwise, they can lead to a cascade of events that may result in loss of independence, some degrees of disability, worsening of quality of life, higher treatment-related costs, and higher mortality. Diniz de Salles et al. aimed to evaluate the association between sarcopenia and frailty in an elderly population admitted to hospital for non-emergency surgical procedures. This is while the association between sarcopenia and frailty is still unclear, while several studies have shown the influence of sarcopenia on frailty over time (1, 3). The secondary objective of the study was to evaluate the correlation of sarcopenia and frailty with postsurgical outcomes. This is mainly because sarcopenia and frailty are believed to have significant adverse effects on the postoperative outcomes (4). In this observational study, frailty was assessed using the modified frailty index (MFI-11). Sarcopenia, on the other hand, was measured through (a) thickness and echogenicity on ultrasound; (b) handgrip strength on dynamometry; and (c) gait speed. They found sarcopenia, in all its domains, was associated with frailty. Unfavorable surgical outcomes were also associated with these two conditions. Diniz de Salles et al. also suggested that screening for sarcopenia and frailty in the elderly patients undergoing elective surgery is relevant, easy to perform, and helps with perioperative risk reduction in this population.

Sarcopenia can be defined by various signs and symptoms, one of which is low muscle strength. There are several tools and methods to diagnose the mass and strength of muscle in sarcopenic people (5) such as handgrip strength test, and dual-energy X-ray absorptiometry (DEXA). Hou et al. in a cohort study with a 600-day follow-up determined the impact of the uremic toxins on the frequency of low handgrip strength in 75 participants divided into three groups: control, chronic kidney disease (CKD), and end-stage renal disease (ESRD). Although, they found a similar rate of sarcopenia between groups, handgrip strength and serum level of indoxyl sulfate, a protein-bound uremic toxin were lower and higher accordingly in ESRD patients. Indoxyl sulfate can impair the function of mitochondria of skeletal muscle cells and muscle anabolism by inducing oxidative stress (6). Moreover, the hospitalization rate was higher in patients with ESRD. They concluded the low handgrip strength to be predictive of hospitalization.

The other articles were focused mainly on osteoporosis. Osteoporosis is a systemic skeletal disease characterized by loss of bone mass and micro architectural integrity that leads to increased bone fragility and risk of fracture (7). With the advancements in science and technology, the quality of life and health status have significantly improved in the past decades. There are still huge differences in the living style, socioeconomic conditions, and medical status, such as smoking, education level, economic disparity, and chronic diseases, in various regions of China (8, 9). Such differences may have contributed to the disparities in the prevalence of osteoporosis in these regions. To address this issue, Wang J. et al. assessed the prevalence of osteoporosis and osteopenia as well as the associated risk factors in the China Community- based Cohort of Osteoporosis (CCCO). The multicenter cross-sectional study was conducted on 19,848 middle-aged and elderly permanent residents of seven representative Chinese regions (10, 11). The bone mineral density at the lumbar vertebrae and hip was determined using the dual-energy X-ray absorptiometry densitometer. Serum levels of bone metabolism markers were also measured. This study revealed dramatic regional differences in the prevalence of osteoporosis in China, with females, those aged 60 or older, with low BMI, low education level, current regular smokers, and with a history of fracture being at a higher risk of osteoporosis. They suggested that more preventive measures and treatment options should be focused on populations with such risk factors.

The maintenance of bone mass is negatively affected by metabolic dysfunctions such as chronic hyperglycemia (12). As a result, osteoporosis is more prevalent among patients with diabetes mellitus. In addition, it is well known that lipid profile disturbances especially high-density lipoprotein cholesterol (HDL-C) and apolipoprotein A1 (APOA1), its major protein component, have key roles in bone mass maintenance through affecting osteoblasts differentiation (13). However, there are controversies regarding their association with bone mineral density (BMD) values. Wang W. et al. investigated Chinese postmenopausal diabetic women for such associations. They found a remarkable association between APOA1 and osteocalcin level (which inhibits bone formation), lumbar BMD, and osteoporosis in contrast to HDL-C.

Cardiovascular disease and osteoporosis are common diseases in older adults, and both are associated with high morbidities (14). Yang and Huang study performed a multivariate logistic regression and stratified analysis to explore the possible relationship between BMD and the risk of CVD in older adults aged over 60 years. This study is expected to provide more guidance on early monitoring and clinical prevention. The cross-sectional study of 2,097 people aged over 60 years speculated that DXA examination or targeted prevention strategies, such as increased sun exposure, appropriate physical exercise, and calcium or vitamin D supplements, should be considered for CVD patients. Meanwhile, for osteoporotic patients or those at high risk of fracture, active anti-osteoporosis drug therapy can increase BMD, improve bone quality, and reduce cardiovascular complications to a certain extent. Negative non-linear relationship was noted between the femur BMD levels and the prevalence of CVD in people aged over 60 years with an inflection point of 0.741 gm/cm^2. No significant differences were found between age, gender, and the comorbidities subgroups. Bone loss therefore was considered as a new risk factor for CVD. This is while future studies are needed to make a comprehensive assessment of combined dietary and biochemical indicators. This also points out the importance of preventive measures for

osteoporosis to indirectly reduce the prevalence of CVD, the world's biggest killer of humans.

Current osteoporosis medications have drawbacks such as possible side effects and having slow onset, therefore developing osteoporosis drugs with faster onset and fewer side effects is essential. Therefore, the Shih et al. study investigated the effects of topical SDTL-E (15) applied for 20 days in ovariectomized (OVX)-induced osteoporosis rat models. The changes in estradiol, various bone turnover markers such as serum alkaline phosphatase (ALP) activity (16), serum and urinary calcium (17), bone mineral density (BMD), various bone mechanics indicators and bone histology were assessed to understand the mechanism of action and the therapeutic efficacy of SDTL-E on osteoporosis. The results demonstrated that the 20-day treatment with topical SDTL-E can improve bone strength and trabecular bone structure in OVX-induced osteoporosis rats. The underlying mechanisms include restoring estradiol levels as well as reducing bone turnover, net bone resorption, bone calcium loss, and calcium excretion through the kidneys. These findings suggest topical application of the plant extract is an efficient potential new approach for rapid treatment of osteoporosis.

One of the most common osteoporotic complications is osteoporotic vertebral fracture (OVF). OVF can lead to loss of height, acute and chronic pain, decreased quality of life, and increased fracture risk (18). Since, falls are known as the main risk factor for fracture in patients with OVF, improving body balance by exercise is believed to have an important role in reducing the future fracture risk (19). Li et al. conducted a systematic review and meta-analysis to assess the effects of resistance and balance exercises in patients with OVF. They found exercising improved quality of life, visual analog pain scale, Timed Up and Go, falls efficacy scale international, kyphosis, and functional reach. These beneficial effects were considerable when training continued for at least 10 weeks.

Odontoid fractures are another relatively common fracture of the spine (C2) vertebral body among the elderly. Despite its prevalence, there are controversies in its treatment. Several studies have suggested surgical management, suggesting it can result in more biochemical stability and fusion compared to the conventional therapies (20). This is while the surgical risks are high among the elderly population. As a result, maintaining the right balance between the risks and benefits of the surgery is challenging. Lenga et al. in a retrospective study with a 5-year follow-up assessed the morbidity and mortality rate of peri- and post-surgery of C1/C2 posterior screw fixation technique against the associated risk factors and mortality. Although they found a high rate of morbidity and mortality in octogenarians, they recommended spine surgery to achieve bone union and preserve of neurological status.

To conclude, all articles of this Research Topic sum up the current information and highlight the current research gaps and elucidate the path for future research on the topic. The findings of the published studies in the current Research Topic have been proposed some acceptable modalities and strategies for management of Osteoporosis, Sarcopenia and Osteosarcopenia, and also improvement of the patients' quality of life.

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Topical application of the plant extract SDTL-E in ovariectomized rats: A potential new approach for treating osteoporosis

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Current osteoporosis medications have drawbacks of causing side effects and having slow onset, therefore developing osteoporosis drugs with faster onset and less side effects is essential. This study investigated the effects of the natural plant extract, SDTL-E, in ovariectomized (OVX)-induced osteoporosis rats. Rats were randomly assigned to sham operation control group (Control Group); OVX rat model group (Model Group) or OVX rat SDTL-E treatment group (SDTL-E Group). All groups underwent ovariectomy, but the Control Group did not have the ovaries removed. SDTL-E Group was treated with SDTL-E, Model and Control Groups were treated with vegetable oil, treatments were topically applied twice daily for 20 days. Results showed when compared with Model Group, SDTL-E Group significantly restored serum estradiol back to near Control Group level, serum ALP activity, serum and urinary calcium were significantly decreased, bone mechanics indicators increased and trabecular bone numbers slightly increased. These results demonstrated 20 days of SDTL-E topical treatment improved bone strength and trabecular bone structure in OVX-induced osteoporosis rats. The underlying mechanisms include restoring estradiol level, reducing bone turnover, net bone resorption, bone calcium loss, and calcium excretion through kidney. These findings suggest topical application of plant extract is a potential new approach with quick efficacy for treating osteoporosis.

KEYWORDS

ovariectomy (OVX), calcium, trabecular bone, bone strength, estradiol, plant extract, topical application, osteoporosis

Introduction

Osteoporosis is a worldwide bone disease having a prevalence of 18.3% (1), affecting around 1.36 billion people out of 7.9 billion global population. Among them, elderly women are at a higher risk. Prevalence of osteoporosis in women over 50 years of age is 37% (2), four times higher than that of men (3). When women reach menopause, estrogen deficiency impairs the normal bone turnover cycle. Decrease in estrogen leads to bone resorption process exceeding bone formation process, resulting in a net loss of bone (4), which increases fracture risk. Osteoporotic fractures are associated with increased mortality rate by 15–20% within 1 year, and a 2.5-fold increased future fracture risk (5). The main objective of treating osteoporosis is to reduce bone loss for lowering fracture risk and to reduce post-fracture mortality risk (6), therefore, it is important for osteoporosis drugs to efficiently lower the fracture rate and post-fracture mortality rate.

Current osteoporosis treatments are mainly administered orally or through injection and there are two types of treatment, the first type is antiresorptive treatment, which decreases the rate of bone resorption, drugs belonging to this type include bisphosphonates, estrogen, and denosumab. The second type is anabolic treatment, which increases bone formation, such as parathyroid hormone analogs (7). The treatment duration of the above osteoporosis drugs can range from 12 to 36 months (8–11), but cannot be taken for a long term due to their various side effects (12–15), seriously decreasing the efficacy of treatment and hindering the reduction of post-fracture mortality risk. Furthermore, a study pointed out that 30% of post-hip fracture deaths occurred within 6 months after fracture (16), indicating that current osteoporosis drugs have serious drawbacks of having a treatment duration much longer than the time for post-fracture deaths to occur. Therefore, it is urgent and essential to develop new osteoporosis drugs with faster onset, shorter treatment duration, better efficacy and less side effects to reduce fracture risk and post-fracture mortality risk.

SDTL-E is a patented natural plant extract (US provisional patent application number: 63/195054). A review pointed out that many natural plants have long been used as herbal medicines to prevent and treat osteoporosis. Main common active ingredients in the plants, such as flavonoids and luteolin, increase osteoblast activity through estrogenic effects, and may also promote osteoblast activity and suppress osteoclast activity through modulating cytokines, and regulating biochemical pathways, such as Wnt/β-catenin, and RANKL/RANK/OPG pathway to achieve their anti-osteoporotic effects (17). Herbal medicines are generally considered natural and safe, however, they may cause hepatotoxicity (17), which is a result of first pass metabolism of the liver when herbal medicine is taken orally (18). To avoid first pass metabolism and hepatotoxicity, topical application route can be considered as it was documented in Ayurveda that the paste of stem of medicinal plants can be applied topically for treatment of osteoporosis (19).

In this study, SDTL-E was topically applied for 20 days to the commonly used ovariectomized (OVX)-induced osteoporosis rat model (20). Then changes of estradiol, various bone turnover markers such as serum alkaline phosphatase (ALP) activity (21), and serum and urinary calcium (22), bone mineral density (BMD), various bone mechanics indicators and bone histology were assessed, so as to understand the mechanism and therapeutic efficacy of SDTL-E on the treatment of osteoporosis.

Materials and methods

Animals and experimental groups

Three-month-old Sprague Dawley female rats were purchased from Guangdong Medical Laboratory Animal Centre (Foshan, Guangdong, China). They were maintained with free access to bottled tap water and were fed with specific-pathogen-free grade whole granular rat feed (Guangdong Medical Laboratory Animal Centre) in a 12-h light–dark cycle under an environment with temperature of 20°C and relative humidity of 80%. Rats were maintained for 15 days before performing further procedures. All procedures performed were approved by the Laboratory Animal Ethics Committee of Jinan University and were in accordance with Animal Care Ethical Guidelines of Jinan University. Rats were randomly assigned to one of the following three treatment groups (10–12 rats per group): (1) sham operation control group (Control Group); (2) OVX rat model group (Model Group); (3) OVX rat model SDTL-E treatment group (SDTL-E Group).

Ovariectomized rat model and treatment

Model Group and SDTL-E Group rats were anesthetized with ethyl ether (Sinopharm Chemical Reagents, Shanghai, China) under sterile environment, then underwent surgery where an incision of approximately 1.5 cm was made on the abdomen 4 cm below the xiphoid. The fat pads were then pulled aside, and the ovaries were removed, followed by repositioning of the fat pads and suturing the incision. The Control Group rats underwent the above surgery but without removing the ovaries. Intramuscular injection of ampicillin (Sinopharm Chemical Reagents) (100,000 units/rat) were then given to the rats for 3 days. The rats were maintained for 3 months, then were weighted and examined by dual-x-ray densitometry to confirm the successful establishment of OVX-induced osteoporosis rat model.

The rats were then anesthetized by injecting 3% pentobarbital sodium (36 mg/kg) (Sinopharm Chemical

Reagents), followed by removing an area of back hair of about 3 cm × 4 cm by smearing with hair removing agent (sodium sulfide 3 g, soap powder 1 g, starch 7 g, add water until paste is formed) on the back for 5 min. After 48 h, 100 μL/kg/day SDTL-E or vegetable oil (Shandong Luhua Group, Yantai, Shandong, China) was topically applied to the hair removed area of the rats twice a day for 20 days. Control Group and Model Group were treated with vegetable oil, while SDTL-E Group was treated with SDTL-E.

Urine was collected for 24 h on day 19 and stored at −20°C for subsequent examination. 45 min after the last treatment, rats were anesthetized by 3% pentobarbital sodium. Blood was extracted from the abdominal aorta and centrifuged at 3,000 rpm, then the supernatant was collected and stored at −80°C for further examination. Rats were then sacrificed by neck dislocation, followed by surgically removing the right femur, tibia and left femur, which were stored at −80°C wrapped with gauze soaked in physiological saline (Sinopharm Chemical Reagents) for further examination.

Serum alkaline phosphatase activity assay

Serum ALP activity was measured by ALP Assay Kit (Nanjing Jiancheung Bioengineering Institute, Nanjing, Jiangsu, China) according to the manufacturer's instructions. Serum ALP in the samples reacted with the substrate, p-nitrophenolphosphate, provided by the kit to generate a yellow product, which was quantified through measuring absorbance at a wavelength of 415 nm by S22PC Spectrophotometer (Shanghai Lengguang Technology Co., Ltd., Shanghai, China) to determine serum ALP activity.

Calcium assay

Serum and urinary calcium ion levels were measured by Calcium Assay Kit (Nanjing Jiancheung Bioengineering Institute) according to the manufacturer's instructions. Calcium ions in the samples reacted with the substrate, Methylthymol blue, provided by the kit to generate a blue product, which was quantified through measuring absorbance at a wavelength of 610 nm by S22PC Spectrophotometer to determine serum and urinary calcium ion levels.

Creatinine assay

Urinary creatinine level was measured by Creatinine Assay Kit (Nanjing Jiancheung Bioengineering Institute) according to the manufacturer's instructions. Urinary creatinine in the samples was oxidized by the oxidase provided by the kit resulting in a purplish red product, which was quantified through measuring absorbance at a wavelength of 546 nm by S22PC Spectrophotometer to determine urinary creatinine level.

Estradiol assay

Serum estradiol level was measured by AxSYM Estradiol Assay (Abbott, Chicago, Illinois, U.S.A) according to the manufacturer's instructions. Serum estradiol in samples were bound to matrix cell as antibody-estradiol-alkaline phosphatase conjugate, followed by reaction with the substrate 4-methylumbelliferyl phosphate to generate fluorescence, which was detected and quantified by ARCHITECT i2000SR immunoassay analyzer (Abbott) to determine serum estradiol level.

Bone densitometry

Rats were anesthetized with ethyl ether. When the rats were in a stable lethargic state for more than 5 min, they were placed under the Lunar Prodigy Dual-X ray Densitometer (GE Healthcare, Chicago, Illinois, U.S.A), and whole-body scan was performed at 60.0 mm/s, with 1.0 × 1.0 mm step size using the calibrated small-animal software to determine the BMD (CV < 1%).

Bone mechanics test

The distal and proximal ends of the right tibia were embedded in polymethyl methacrylate and then mounted onto a combined-axial-motion and torsional-testing jig, which was attached to the 855 Mini Bionix testing system (MTS Systems, Eden Prairie, Minnesota, U.S.A) for bone torsional testing. The distal end of the specimen was rotated laterally at 6°/min until bone failure was observed. The load displacement curves were recorded and the following parameters were calculated: torsion power (N), torque (Nmm), shear stress (MPa), and shear modulus (MPa).

Histological examination

All the soft tissues of the proximal half of the left femur were removed, freshly prepared 10% neutral buffered formalin (Sinopharm Chemical Reagents) was used to fix the bone for 24 h. The bone was then rinsed with 0.01 M phosphate buffered saline (Sinopharm Chemical Reagents). After that, the bone was decalcified by 20% formic acid (Sinopharm Chemical Reagents) for 6 days, with the formic acid changed daily. Then the bone was rinsed with 0.01 M phosphate buffered saline,

TABLE 1 BMD of rats 3 months after ovariectomy before treatment by bone densitometry analysis.

Groups	(*n*)	BMD (g/cm^2)
Model	12	0.20 ± 0.02*
Control	12	0.22 ± 0.02
SDTL-E	10	0.20 ± 0.01*

Values are mean ± *SD*.
*$p < 0.05$ vs. Control Group.

and dehydrated with 75, 80, 95, and 100% ethanol (Sinopharm Chemical Reagents) successively, followed by treating 15 min in xylene (Sinopharm Chemical Reagents) twice. After that, the bone was soaked in a 60°C paraffin bath for 3 h and embedded overnight. On the next morning, 5 μm thick cross sections were prepared on a polylysine-treated glass slide. The glass slide was dried, followed by hematoxylin and eosin staining (Sinopharm Chemical Reagents) and changes in bone structure were examined under a light microscope (Leica, Wetzlar, Germany).

Statistical analysis

All data were analyzed by the statistical software GraphPad Prism 9 (GraphPad Software Inc., La Jolla, CA, U.S.A.). Results are expressed as mean ± standard deviation (*SD*). Multiple comparisons between groups were analyzed by one-way ANOVA with Newman–Keuls *post-hoc* test and were considered statistically significant when $p < 0.05$.

Results

Reduced bone mineral density in ovariectomized-induced osteoporosis rat model

Three months after Model Group and SDTL-E Group rats were OVX, BMD was measured before treatment. The BMD of Model Group and SDTL-E Group were significantly lower than the Control Group ($p < 0.05$) (Table 1), indicating the OVX-induced osteoporosis rat model was successfully established.

Body weight changes of ovariectomized-rats

Rats of all groups had similar body weight before ovariectomy. The body weight of Model Group and SDTL-E Group were significantly higher than the Control Group ($p < 0.01$) after treatment day 1, which was 3 months after ovariectomy. SDTL-E treatment on SDTL-E Group, and vegetable oil treatment on Model Group and Control Group for 20 days had no significant effects on body weights of different group rats, with the body weight of Model Group and SDTL-E Group still significantly higher than the Control Group ($p < 0.01$) (Table 2 and Figure 1).

SDTL-E treatment reduced serum alkaline phosphatase activity of ovariectomized-induced osteoporosis rats

ALP assay was performed to evaluate the effect of SDTL-E on the bone turnover biomarker, serum ALP activity. Compared to Control Group with serum ALP activity of 19.41 ± 5.76 King-Armstrong unit (K.A.U)/100 mL, Model Group had a 140.54% significantly higher serum ALP activity of 46.69 ± 13.32 K.A.U/100 mL ($p < 0.01$). After 20 days of SDTL-E treatment, serum ALP activity of SDTL-E Group rats was 19.24 ± 6.06 K.A.U/100 mL, significantly lower than that of the Model Group by 58.79% ($p < 0.01$) and was similar to that of the Control Group (Figure 2), indicating topical treatment of SDTL-E for 20 days can significantly regulate and reduce serum ALP activity in OVX-induced osteoporosis rats.

SDTL-E treatment increased serum estradiol level of ovariectomized-induced osteoporosis rats

Estradiol assay was performed to evaluate the effect of SDTL-E on serum estradiol level. Compared to Control Group with serum estradiol level of 27.27 ± 6.77 pg/mL, Model Group had a 26% significantly lower serum estradiol level of 20.18 ± 4.77 pg/mL ($p < 0.05$). After 20 days of SDTL-E treatment, serum estradiol level of SDTL-E Group rats was 25.30 ± 4.52 pg/mL, significantly higher than that of the Model Group by 25.37% ($p < 0.05$) and was similar to that of the Control Group (Figure 3), indicating topical treatment of SDTL-E for 20 days can significantly increase and restore serum estradiol level in OVX-induced osteoporosis rats.

SDTL-E treatment reduced serum and urinary calcium ion levels of ovariectomized-induced osteoporosis rats

Calcium assay was performed to evaluate the effects of SDTL-E on the bone turnover biomarkers, serum and

TABLE 2 Body weight of rats before ovariectomy and after treatment.

Groups	(*n*)	Body weight before ovariectomy (g)	Body weight after treatment day 1 (g)	Body weight after treatment day 20 (g)
Model	12	266.00 ± 10.37	341.92 ± 34.36**	343.00 ± 34.93**
Control	12	268.58 ± 11.02	289.08 ± 21.04	291.25 ± 21.78
SDTL-E	10	264.80 ± 8.16	346.00 ± 29.62**	341.10 ± 28.51**

Values are mean ± SD.
**$p < 0.01$ vs. Control Group.

urinary calcium ion levels. Compared to Control Group with serum calcium ion level of 2.28 ± 0.20 mM, Model Group had a 18.42% significantly higher serum calcium ion level of 2.70 ± 0.32 mM ($p < 0.01$). After 20 days of SDTL-E treatment, serum calcium ion level of SDTL-E Group rats was 1.94 ± 0.21 mM, significantly lower than that of the Model Group by 28.15% ($p < 0.01$) and was also lower than that of the Control Group ($p < 0.01$) (Figure 4A), indicating topical treatment of SDTL-E for 20 days can significantly stop bone calcium loss and the transfer of calcium ions to the blood in OVX-induced osteoporosis rats.

Compared to Control Group with urinary calcium ion level of 1.39 ± 0.79 mM, Model Group had a 76.26% significantly higher urinary calcium ion level of 2.45 ± 0.76 mM ($p < 0.01$). After 20 days of SDTL-E treatment, urinary calcium ion level of SDTL-E Group rats was 1.76 ± 0.80 mM, significantly lower than that of the Model Group by 28.16% ($p < 0.01$) and was similar to that of the Control Group (Figure 4B), indicating topical treatment of SDTL-E for 20 days can significantly stop the loss and transfer of calcium ions to the urine in OVX-induced osteoporosis rats.

SDTL-E treatment had no significant effects on urinary calcium/creatinine ratio of ovariectomized-induced osteoporosis rats

Calcium assay and creatinine assay were performed to quantify urinary calcium and creatinine to calculate urinary calcium/creatinine ratios in different rat groups. Compared to Control Group with urinary calcium/creatinine ratio of 0.36 ± 0.33, Model Group had a 36.11% higher urinary calcium/creatinine ratio of 0.49 ± 0.29, but the difference was not statistically significant. After 20 days of SDTL-E treatment, urinary calcium/creatinine ratio of SDTL-E Group rats was 0.36 ± 0.14, which was similar to that of the Control Group and was lower than that of the Model Group, but the difference was not statistically significant (Figure 5).

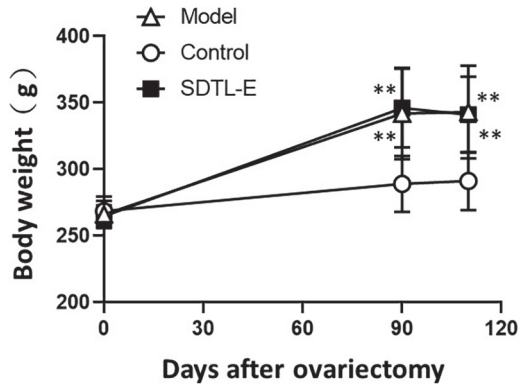

FIGURE 1
Changes of body weight of rats. 90 days after ovariectomy, Model Group ($n = 12$) and Control Group ($n = 12$) rats were treated with vegetable oil. SDTL-E Group ($n = 10$) rats were treated with SDTL-E. All treatments were topically applied twice daily for 20 days. Rats were weighed before ovariectomy, 90 days after ovariectomy (treatment day 1) and 110 days after ovariectomy (treatment day 20). Values are mean ± *SD*.
**$p < 0.01$ vs. Control Group.

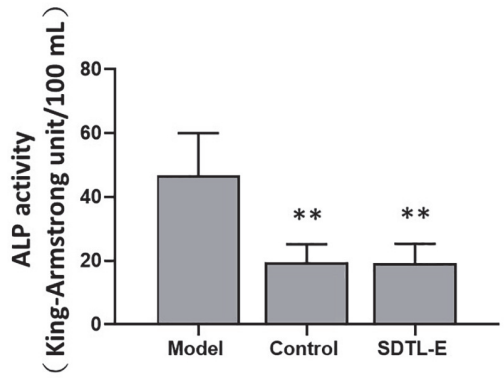

FIGURE 2
SDTL-E treatment reduced serum ALP activity of OVX-induced osteoporosis rats. Model Group ($n = 11$) and Control Group ($n = 10$) rats were treated with vegetable oil. SDTL-E Group ($n = 10$) rats were treated with SDTL-E. All treatments were topically applied twice daily for 20 days. Serum ALP activities were determined by ALP assay. Values are mean ± *SD*.
**$p < 0.01$ vs. Model Group.

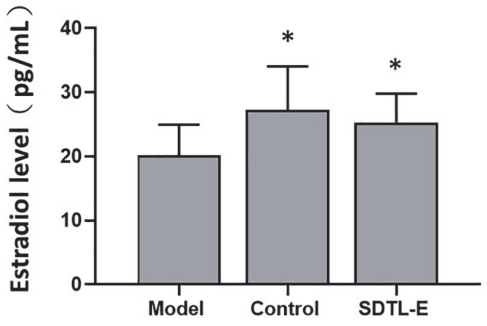

FIGURE 3
SDTL-E treatment increased serum estradiol level of OVX-induced osteoporosis rats. Model Group ($n = 11$) and Control Group ($n = 11$) rats were treated with vegetable oil. SDTL-E Group ($n = 10$) rats were treated with SDTL-E. All treatments were topically applied twice daily for 20 days. Serum estradiol levels were determined by estradiol assay. Values are mean ± *SD*. $^{*}p < 0.05$ vs. Model Group.

SDTL-E treatment had no significant effects on bone mineral density of ovariectomized-induced osteoporosis rats

Bone densitometry was performed on the right femur to evaluate the effects of SDTL-E on BMD. Compared to Control Group with BMD of 0.22 ± 0.02 g/cm^2, Model Group had a 9.09% significantly lower BMD of 0.20 ± 0.01 g/cm^2 ($p < 0.01$). After 20 days of SDTL-E treatment, BMD of SDTL-E Group rats was 0.20 ± 0.01 g/cm^2, which had no significant difference with the Model Group (Figure 6), indicating topical treatment of SDTL-E for 20 days has no significant effect on BMD in OVX-induced osteoporosis rats.

SDTL-E treatment increased bone strength of ovariectomized-induced osteoporosis rats

Bone torsional testing results showed shear stress of Model Group was significantly lower than that of the Control Group ($p < 0.05$) (Supplementary Table 1 and Figure 7C). Torsion power, torque and shear modulus of Model Group were lower than that of the Control Group, but the differences were not statistically significant (Supplementary Table 1 and Figures 7A,B,D). Compared to Model Group with shear stress of 178.281 ± 45.672 Mpa, shear stress of SDTL-E Group rats after 20 days of SDTL-E treatment was 249.502 ± 63.445 Mpa, significantly higher than that of the Model Group by 39.93% ($p < 0.05$) and was similar to the shear stress of the Control Group of 235.540 ± 58.097 Mpa (Supplementary Table 1 and Figure 7C). Torsion power, torque and shear modulus of

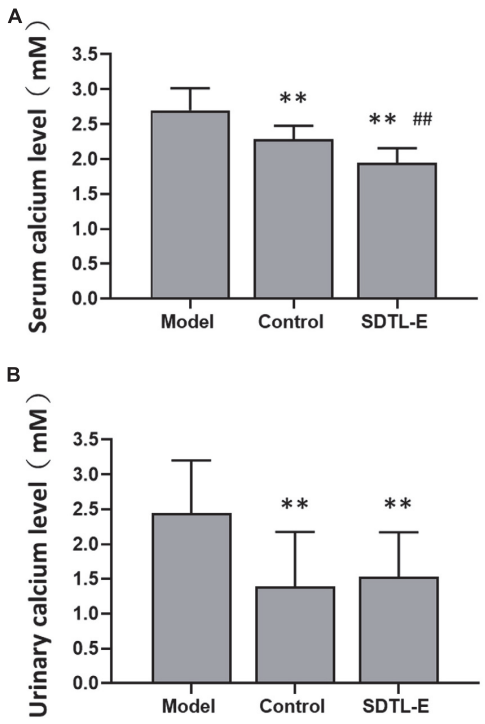

FIGURE 4
SDTL-E treatment reduced serum and urinary calcium ion levels of OVX-induced osteoporosis rats. Model Group ($n = 11–12$) and Control Group ($n = 10–11$) rats were treated with vegetable oil. SDTL-E Group ($n = 10$) rats were treated with SDTL-E. All treatments were topically applied twice daily for 20 days. Calcium ion levels in **(A)** serum and **(B)** urine were determined by calcium assay. Values are mean ± *SD*. $^{**}p < 0.01$ vs. Model Group. $^{\#\#}p < 0.01$ vs. Control Group.

SDTL-E Group were similar to that of the Control Group and were higher than that of the Model Group but the differences were not statistically significant (Supplementary Table 1 and Figures 7A,B,D). These results indicate that topical treatment of SDTL-E for 20 days can increase bone strength in OVX-induced osteoporosis rats.

SDTL-E treatment improved the structure of osseous tissue of ovariectomized-induced osteoporosis rats

Figure 8A shows a clear structure of osseous tissue of the Control Group rats, the trabecular bones were thick, darkly stained and densely aligned, the interspaces of trabeculae were small and filled with red bone marrow indicating active blood production. Figure 8B shows the structure of osseous tissue of the Model Group rats, when compared with the Control Group rats, the number of trabecular bones decreased, the trabecular bones were lightly stained, shrunk, fractured and

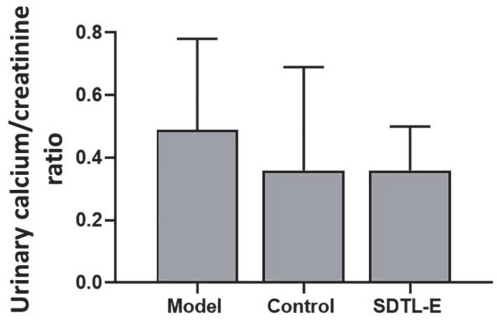

FIGURE 5
SDTL-E treatment had no significant effects on urinary calcium/creatinine ratio of OVX-induced osteoporosis rats. Model Group ($n = 12$) and Control Group ($n = 11$) rats were treated with vegetable oil. SDTL-E Group ($n = 10$) rats were treated with SDTL-E. All treatments were topically applied twice daily for 20 days. Urinary calcium/creatinine ratio was calculated by results obtained from calcium assay and creatinine assay. Values are mean ± *SD*.

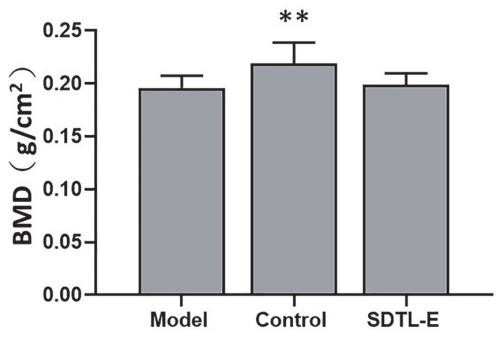

FIGURE 6
SDTL-E treatment had no significant effects on BMD of OVX-induced osteoporosis rats. Model Group ($n = 12$) and Control Group ($n = 11$) rats were treated with vegetable oil. SDTL-E Group ($n = 10$) rats were treated with SDTL-E. All treatments were topically applied twice daily for 20 days. BMD was determined by performing bone densitometry evaluation on the right femur. Values are mean ± *SD*. $^{**}p < 0.01$ vs. Model Group.

sparse, the interspaces of trabeculae were widened, fatty tissue increased with fewer red bone marrow indicating low blood production. **Figure 8C** shows the structure of osseous tissue of the SDTL-E Group rats, when compared with the Control Group rats, the number of trabecular bones decreased, the trabecular bones were lightly stained, shrunk, fractured and sparse, the interspaces of trabeculae were widened, fatty tissue increased with fewer red bone marrow indicating low blood production, however, when compared with the Model Group rats, the number of trabecular bones and trabecular bone thickness showed a slight increase, studies with longer duration will be needed to investigate longer term effects of SDTL-E on osseous tissue structure of OVX-induced osteoporosis rats.

Discussion

In postmenopausal osteoporosis, estrogen deficiency leads to increased bone turnover, which can be indicated by an elevation of the bone turnover marker, serum ALP activity (23). Despite an overall increase in bone turnover, bone resorption exceeds bone formation as indicated in a study that menopause induced 37–52% and 79–97% increase in bone formation and bone resorption marker levels, respectively (24), resulting in increased net bone resorption, which is the main determinant of high serum calcium levels (25). Lowered estrogen levels also lead to increased calcium excretion through kidney and decreased intestinal absorption of calcium (26), which stimulates bone calcium release into blood (25). These factors above lead to observations that the bone turnover markers serum calcium, urinary calcium and urinary calcium/creatinine are elevated in postmenopausal osteoporosis patients (27, 28). Results of this study showed topical treatment of SDTL-E for 20 days on SDTL-E Group rats resulted in significantly higher estradiol, lower serum ALP, lower serum calcium and lower urinary calcium levels when compared to Model Group rats. These results suggest that SDTL-E can regulate and restore estradiol levels to reduce bone turnover, net bone resorption, bone calcium loss and excretion of calcium through kidney in OVX-induced osteoporosis rats.

Apart from being a bone turnover marker, serum ALP is also a marker for bone formation by osteoblasts (24). SDTL-E lowering serum ALP indicated suppression of bone turnover and bone formation by osteoblasts, and SDTL-E lowering serum calcium level indicated a decrease in net bone resorption, these observations indirectly reflect that SDTL-E also suppressed bone resorption by osteoclasts and this suppression effect is greater than that on bone formation by osteoblasts in OVX-induced osteoporosis rats. However, the results did not individually evaluate how bone formation and bone resorption were affected by SDTL-E. Future studies with evaluation of changes on bone formation markers, such as serum osteocalcin, and bone resorption markers, such as serum carboxyterminal cross-linked telopeptide of type I collagen (CTX), and analysis of osteoclast activation by tartrate-resistant acid phosphatase (TRAP) staining can be done to give a full picture on how SDTL-E reduces net bone resorption.

Estradiol is the most common and biologically active form of estrogen in mammals. Apart from the ovaries, estradiol is also produced in various extragonadal organs, such as the pancreas, brain, adrenal glands, skin and adipose tissue (29, 30). It is important to note that high levels of estrogen not only increase the risk of developing breast and endometrial cancer (15), but can also lead to side effects such as thrombosis (31). The results of this study suggest, SDTL-E, which consists of only plant extracts, probably stimulated estradiol production from the above-mentioned organs to endogenously restore estradiol levels in OVX-induced osteoporosis rats back to normal levels

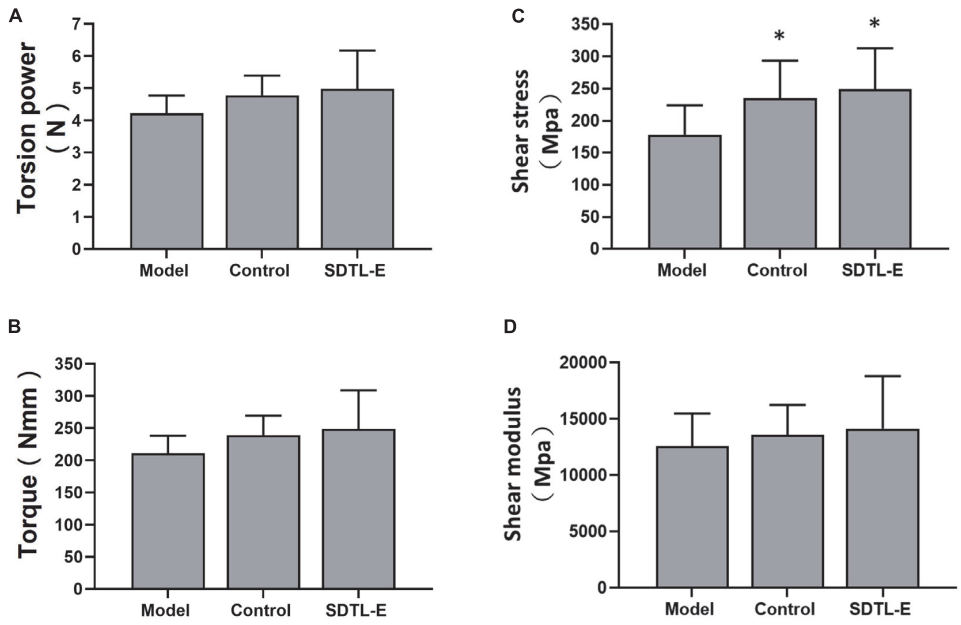

FIGURE 7
SDTL-E treatment increased bone strength of OVX-induced osteoporosis rats. Model Group ($n = 9$) and Control Group ($n = 11$) rats were treated with vegetable oil. SDTL-E Group ($n = 9$) rats were treated with SDTL-E. All treatments were topically applied twice daily for 20 days. Bone mechanics parameters, **(A)** torsion power **(B)** torque **(C)** shear stress and **(D)** shear modulus were determined by bone torsional testing. Values are mean ± *SD*. $^*p < 0.05$ vs. Model Group.

similar to that of Control Group rats, therefore minimizing the risks of causing side effects related to high estrogen levels.

Bone strength is positively correlated to bone's ability to resist fracture (32). The commonly used marker of bone strength, BMD (33), as well as bone mechanics indicators from torsional testing, were assessed in this study to investigate SDTL-E effect on bone strength of OVX-rats. BMD of Model Group OVX-rats was significantly lower than that of Control Group rats, indicating successful establishment of the OVX-induced osteoporosis rat model. Bone torsional experiments showed SDTL-E treatment on SDTL-E Group rats resulted in significantly higher shear stress compared to Model Group, torsion power, torque and shear modulus of SDTL-E Group rats were also increased and restored to similar levels to that of Control Group rats, indicating that topical treatment of SDTL-E can restore bone strength of OVX-rats back to near normal level in 20 days. On the other hand, treatment of SDTL-E for 20 days on SDTL-E Group rats did not have significant effects on BMD. This could be due to the duration of the study is much shorter than the time needed for mineralization of newly formed bone, which can take up to around 30 months (32). However, that does not indicate SDTL-E cannot improve bone strength as BMD only accounts for 50–70% of the variation in bone strength (32, 34).

BMD and bone structure both contribute to the strength of the skeleton (32). The early stage of the bone remodeling process starts from the trabecular bone. The number of trabecular bones, trabecular bone thickness and the degree of connectivity, which are decreased in osteoporosis, influence the mechanical strength of the bone (33). SDTL-E treatment on OVX-rats for 20 days restored bone strength but not BMD back to normal levels, suggesting that SDTL-E increased bone strength of OVX-rats through mechanisms other than restoring BMD. Microscopic observations from this study demonstrated Model Group and SDTL-E Group rats had decreased, shrunk, fractured and sparse trabecular bone structure compared to Control Group rats, however, number of trabecular bones in SDTL-E Group rats was greater than that in Model Group rats, indicating that topical treatment of SDTL-E for 20 days can already increase bone strength of OVX-rats through improving trabecular bone number and thickness. Despite the calcium content is low in trabecular bone, the increase in number of trabecular bones in SDTL-E Group rats can also be accounted for the reduction of serum calcium level by SDTL-E as calcium is required for trabecular bone formation (35). While current bone histology results provided qualitative analysis on observable changes of trabecular bone structure, future experiments with more in-depth analysis using imaging software to measure bone histomorphometric data of stained trabecular tissues or employing micro computed X-ray tomography (microCT) to evaluate the 3D bone microstructure can be done to accurately quantify the improvements of trabecular bone structure by SDTL-E treatment.

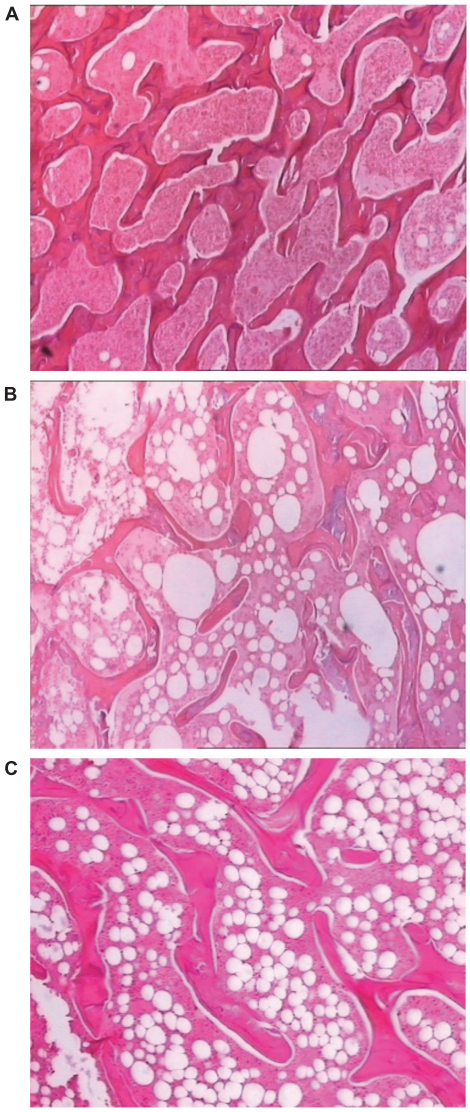

FIGURE 8
SDTL-E treatment improved the structure of osseous tissue of OVX-induced osteoporosis rats. Model Group and Control Group rats were treated with vegetable oil. SDTL-E Group rats were treated with SDTL-E. All treatments were topically applied twice daily for 20 days. Osseous tissues of left femur were stained by hematoxylin and eosin, followed by observation under light microscope with 200X magnification. Above are representative staining images of **(A)** Control Group **(B)** Model Group and **(C)** SDTL-E Group rats.

Osteoporotic fractures can lead to increased mortality rate (5), which might have been caused by post-fracture complications. The major complications related to post-fracture deaths include cardiac, respiratory, cerebrovascular, and malignancy diseases (16). The study results showed topical treatment of SDTL-E increased and restored bone strength in 20 days, indicating SDTL-E can prevent fractures and therefore reduce post-fracture mortality rate. Histological observations showed Model Group OVX-rats had significantly increased body weight, decreased trabecular bone number, increased fatty tissue and decreased blood producing red bone marrow between interspaces of trabeculae when compared to Control Group rats. Previous studies showed estrogen deficiency in post-menopause can lead to obesity and increased fat in bone marrow (36), positive correlation between blood cell count and BMD in post-menopausal women (37), and anemia is associated with increased risk of osteoporosis and fracture risk (38, 39). Combined observations from the above studies and the current study suggest that estrogen deficiency leading to obesity and increased fatty tissue between interspaces of trabeculae resulting in anemia is a possible mechanism for developing osteoporosis in OVX-rats. SDTL-E treatment for 20 days on SDTL-Group rats restored estradiol level, bone strength and trabecular bone structure, but did not decrease body weight or increase blood producing red bone marrow. Contrary observations were found in previous studies demonstrating that estrogen can regulate body weight (40) and increase red blood cell production (41). It was a drawback of this study to have not quantitatively monitored the amount of blood producing red bone marrow and therefore could not assess the effect of restored estradiol level on the changes of blood producing red bone marrow, which can be investigated in future experiments with longer study duration and quantification of red bone marrow by dual-energy computed tomography (42), or through measuring the amount of hematopoietic cellular constituents in bone marrow by flow cytometry (43).

Some studies have shown that other osteoporosis drugs administered through oral gavage or injection in OVX-induced osteoporosis rats require longer time to restore bone strength than topical application of SDTL-E, and their efficacy may not be significant. For example, oral gavage of the bisphosphonate drug, Alendronate Sodium, took 6 months (~180 days) to significantly increase and restore bone strength back to normal level (44). Injection of the parathyroid hormone analog drug, Forteo®, took 12 weeks (~84 days) to significantly increase and restore bone strength back to normal level (45). Oral gavage of the selective estrogen receptor modulator drug, Raloxifene for 4 weeks (~28 days) did not significantly increase bone strength (46). For other herbal preparation or plant extracts, studies on their topical application effects on bone strength in OVX-rats are lacking, but reports have shown they can restore bone strength in OVX-induced osteoporosis rats after oral administration for a period ranging from 4 weeks (~28 days) (47) to 26 weeks (~182 days) (48). This study demonstrated that SDTL-E can treat osteoporosis in OVX-rats by increasing bone strength, number of trabecular bones and trabecular bone thickness. Its possible mechanism is through stimulation of endogenous regulation and restoration of estradiol back to normal level to reduce bone turnover, net bone resorption, bone calcium loss and excretion of calcium through kidney. The above-mentioned therapeutic effects can be achieved by topical

application of SDTL-E for 20 days, which is shorter than the time required by the above-mentioned osteoporosis drugs, herbal preparation or plant extracts to restore bone strength (44–48), and much shorter than the 6 months-time for post-fracture deaths to occur reported in other studies (16). Therefore, SDTL-E can effectively treat osteoporosis and restore bone strength for preventing fractures, lowering fracture rate and post-fracture mortality rate, improving the clinical efficacy for osteoporosis.

While this study demonstrated the effects of SDTL-E in treating OVX-induced osteoporosis rats, improvements can be done to the study design by adding a Control Group with SDTL-E treatment, a Positive Control Group with a known osteoporosis drug treatment and SDTL-E Treatment Groups with different SDTL-E dosages. These improvements can be implemented in future studies to provide valuable information on SDTL-E effects on bones of normal rats or participants, how SDTL-E performs when directly compared to other anti-osteoporotic drugs and the optimal dosage of SDTL-E to be used in treating osteoporosis.

The current study findings suggest topical application of SDTL-E has the advantages of fast and obvious therapeutic effects, and has potential to be developed into osteoporosis drug with quick efficacy and minimal side effects. These findings may overthrow the common perception that the onset time of topical drugs are slower than that of oral and injection drugs, providing new insights for future drug research.

Conclusion

Topical treatment of SDTL-E for 20 days improved bone strength and trabecular bone structure in OVX-induced osteoporotic rats. The underlying mechanisms include restoring estradiol back to normal level, reducing bone turnover, net bone resorption, bone calcium loss and excretion of calcium through kidney. These results suggest SDTL-E has the potential to be developed into osteoporosis drug with quick efficacy and minimal side effects, and topical application of natural plant extract is a possible new approach for treatment of osteoporosis.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author/s.

Ethics statement

The animal study was reviewed and approved by the Laboratory Animal Ethics Committee of Jinan University and was performed in accordance with Animal Care Ethical Guidelines of Jinan University.

Author contributions

H-YS, J-HL, and A-HX: conceptualization and methodology. H-YS, J-HL, A-HX, and BW: data analysis. JT: funding acquisition. J-HL and A-HX: investigation. H-YS: supervision. BW: writing—original draft. H-YS, JT, and BW: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

Authors JT and BW were employed by the SDTL Reborntech Company Limited.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.988235/full#supplementary-material

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Indoxyl sulfate mediates low handgrip strength and is predictive of high hospitalization rates in patients with end-stage renal disease

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Background and aims: Sarcopenia has a higher occurrence rate in patients with chronic kidney disease (CKD) and end-stage renal disease (ESRD) than in the general population. Low handgrip strength—and not sarcopenia *per se*—is associated with clinical outcomes in patients with CKD, including cardiovascular mortality and hospitalization. The factors contributing to low handgrip strength are still unknown. Accordingly, this study aimed to determine whether uremic toxins influence low handgrip strength in patients with CKD.

Materials and methods: This cohort study lasted from August 2018 to January 2020. The participants were divided into three groups: the control group [estimated glomerular filtration rate (eGFR) $\geq$ 60 ml/min], an advanced CKD group (eGFR = 15–60 ml/min), and an ESRD group (under maintenance renal replacement therapy). All participants underwent handgrip strength measurement, dual-energy X-ray absorptiometry, and blood sampling for myokines (irisin, myostatin, and interleukin 6) and indoxyl sulfate. Sarcopenia was defined according to the Asian Working Group for Sarcopenia consensus as low appendicular skeletal muscle index (appendicular skeletal muscle/height^2 of $<$ 7.0 kg/m^2 in men and $<$ 5.4 kg/m^2 in women) and low handgrip strength ($<$ 28 kg in men and $<$ 18 kg in women).

Results: Among the study participants (control: n = 16; CKD: n = 17; and ESRD: n = 42), the ESRD group had the highest prevalence of low handgrip strength (41.6 vs. 25% and 5.85% in the control and CKD groups, respectively; $p < 0.05$). The sarcopenia rate was similar among the groups (12.5, 17.6, and 19.5% for the control, CKD, and ESRD groups, respectively; p = 0.864). Low handgrip strength was

associated with high hospitalization rates within the total study population during the 600-day follow-up period (p = 0.02). The predictions for cardiovascular mortality and hospitalization were similar among patients with and without sarcopenia (p = 0.190 and p = 0.094). The serum concentrations of indoxyl sulfate were higher in the ESRD group (227.29 ± 92.65 μM vs. 41.97 ± 43.96 μM and 6.54 ± 3.45 μM for the CKD and control groups, respectively; $p < 0.05$). Myokine concentrations were similar among groups. Indoxyl sulfate was associated with low handgrip strength in univariate and multivariate logistic regression models [univariate odds ratio (OR): 3.485, 95% confidence interval (CI): 1.372–8.852, p = 0.001; multivariate OR: 8.525, 95% CI: 1.807–40.207, p = 0.007].

Conclusion: Handgrip strength was lower in the patients with ESRD, and low handgrip strength was predictive of hospitalization in the total study population. Indoxyl sulfate contributed to low handgrip strength and counteracted the benefits of myokines in patients with CKD.

KEYWORDS

indoxyl sulfate, irisin, chronic kidney disease, sarcopenia, handgrip strength, frailty

1. Introduction

Chronic kidney disease (CKD) is defined as impaired glomerular filtration caused by structural or chronic damage to the glomerulus and the genitourinary tract. The risk factors for CKD include advanced age, metabolic disorders such as diabetes mellitus, uncontrolled hypertension, autoimmune disorders such as systemic lupus erythematous, and hereditary disorders such as polycystic kidney disease (1). The progressive decrease in glomerular filtration rate (GFR) can result in multiple complications, such as the activation of the renin–angiotensin–aldosterone system, insulin resistance, secondary hyperparathyroidism, vitamin D deficiency, electrolyte imbalance, and the accumulation of uremic toxins, which can trigger further comorbidities (2–4). Patients with CKD and end-stage renal disease (ESRD) are more vulnerable to comorbidities such as uncontrolled congestive heart failure, fluid overload, altered consciousness, decreased erythropoiesis, renal osteodystrophy, sarcopenia, and frailty; therefore, these patients have a higher incidence of hospitalization or mortality than the general population (5–8).

Frailty is defined as the state of increased vulnerability resulting from aging-associated decline with compromised coping ability for daily or acute stressors due to loss in reserve and function across multiple physiologic systems (9). Sarcopenia, a major component of frailty, is defined as age-related loss of muscle mass, plus low muscle strength, and/or low physical performance. (10). The intrinsic contraction–extension pattern of skeletal muscle supports the posture and structure of the body. Sarcopenia is diagnosed either through physical assessments, such as a handgrip strength test, or by using radiology tools such as dual-energy X-ray absorptiometry (DEXA) (11). Myokines such as irisin or myostatin are released from the skeletal muscle, and they play a role in the modulation of skeletal muscle homeostasis and by extension the development of sarcopenia and frailty (12). Patients with CKD are more susceptible to frailty or sarcopenia due to advanced age with other comorbidities such as insulin resistance or metabolic acidosis, and dysregulation of anabolic myokines (13, 14). Sarcopenia diagnosed through physical assessment, as opposed to radiology, was associated with poorer clinical outcomes in patients with CKD. However, the mechanisms behind this relationship are still under investigation (15).

Indoxyl sulfate is a protein-bound uremic toxin found in patients with CKD. The accumulation of this uremic toxin could directly increase the oxidative stress levels within skeletal muscle cells and impair the function of mitochondria (16). Because skeletal muscle is part of the cardiovascular system, this could affect catabolism and accelerate the development of frailty and sarcopenia (17, 6). Furthermore, indoxyl sulfate influenced decrease skeletal muscle anabolism, resulting in atrophy (17). For patients undergoing dialysis, handgrip strength is related to frailty, and in patients with CKD, handgrip strength is related to specific comorbidities or mortality (18, 19). However, few clinical studies have researched the relationship of uremic toxins with sarcopenia and frailty or the interaction between myokines and body composition. Accordingly, the present study investigated the role of myokines in contributing to low handgrip strength in patients with CKD and ESRD.

2. Materials and methods

2.1. Ethics and study protocol

This study was conducted at a regional hospital in New Taipei City, Taiwan, in accordance with the tenets of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Human Studies at Cardinal Tien Hospital (CTH-107-3-5-027). The study period lasted from August 2018 to January 2020. The inclusion criteria were as follows: (a) estimated GFR (eGFR) < 60 ml/min or spot urine proteinuria > 200 mg/g, (b) age > 20 years, and (c) able to communicate verbally in Mandarin Chinese. The exclusion criteria were as follows: having unstable angina, acute myocardial infarction during the past

6 months, severe anemia (Hb < 8 g/dL), systolic hypertension (> 190 mmHg), active inflammation or infection, malignant cancer, autoimmune diseases, emotional instability, musculoskeletal disability, uncontrolled cardiac failure or respiratory problems, or being hospitalized during the past month. After the participants were enrolled, they were divided into three groups: (1) the control group (eGFR > 60 ml/min), (2) the CKD group (eGFR 15–60 ml/min), and (3) the ESRD group. ESRD was defined as receiving maintenance renal replacement therapy continuously for > 3 months. For the ESRD patients, the participants received hemodialysis (three times per week with duration 3–4 h for each session) with polyethersulfone as the dialyzer materials. The Kt/V

TABLE 1 Patient demographics by group.

	Control	CKD	ESRD	*p*-value
Sample size	16	17	41	
Age*	48.5 ± 10.94	65.64 ± 5.93	60.68 ± 14.81	$p < 0.05$
Female (%)	12 (75)	4 (23.5)	14 (34.1)	$P < 0.05$
Diabetes mellitus (%)*	1 (6.25)	11 (64.7)	26 (64.9)	$p < 0.05$
Hypertension (%)*	3 (18.75)	14 (82.35)	36 (85.71)	$p < 0.05$
Coronary artery disease (%)	0 (0)	1 (5.89)	4 (9.52)	$p = 0.425$
Congestive heart failure (%)&	0 (0)	1 (5.89)	17 (40.47)	$p < 0.05$
Malignancy (%)	0 (0)	1 (5.89)	1 (2.23)	$p = 0.569$

*Control vs. CKD; &Control vs. ESRD.

TABLE 2 Incidence of low handgrip strength and sarcopenia by group.

	Control	CKD	ESRD	*p*-value
Sample size	16	17	41	
Body mass index [kg/height (meter)^2]	25.95 ± 4.79	24.12 ± 6.68	25.73 ± 3.44	$P = 0.398$
Grasping power (kg)&^	28.95 ± 9.64	31.63 ± 8.47	21.30 ± 9.43	$p < 0.05$
Case number of low handgrip strength (percentage)	4 (25)	1 (5.85)	17 (41.46)	$p < 0.05$
Case number of sarcopenia based on AWGS algorithm (percentage)	2 (12.5)	3 (17.6)	8 (19.5)	$P = 0.804$

*Control vs. CKD; &Control vs. ESRD; ^CKD vs. ESRD.

TABLE 3 Hematological and biochemical results by group.

	Control	CKD	ESRD	*p*-value
Blood urea nitrogen (mg/dL)*&^	16.93 ± 9.25	44.91 ± 26.85	65.59 ± 21.44	$p < 0.05$*
Creatinine (mg/dL)*&^	0.76 ± 0.22	3.13 ± 2.06	9.70 ± 3.31	$p < 0.05$*
Estimated glomerular filtration rate (ml/min)*&^	97.77 ± 17.64	35.38 ± 25.75	7.45 ± 11.12	$p < 0.05$*
Sodium (mEq/L)&^	140.13 ± 2.39	140.73 ± 3.80	138.10 ± 2.54	$p < 0.05$*
Potassium (mEq/L)	4.17 ± 0.29	4.61 ± 0.62	4.35 ± 0.73	$p = 0.156$
Calcium (mg/dL)	9.61 ± 0.62	9.11 ± 0.70	9.09 ± 0.81	$p = 0.081$
Phosphorus (mg/dL)&^	3.81 ± 0.51	4.22 ± 0.72	5.44 ± 1.81	$p < 0.05$*
Uric acid (mg/dL)	5.71 ± 1.33	6.99 ± 1.58	6.35 ± 1.75	$p = 0.115$
Alkaline phosphatase (mg/dL)	68.87 ± 18.52	74.33 ± 28.77	80.53 ± 28.18	$p = 0.313$
Albumin (g/dL)&	4.33 ± 0.42	4.23 ± 0.32	4.03 ± 0.34	$p < 0.05$*
Cholesterol (mg/dL)	170.20 ± 27.78	143.73 ± 41.09	152.33 ± 39.61	$p = 0.148$
Triglyceride (mg/dL)	135.18 ± 54.59	118.82 ± 53.00	142.97 ± 69.62	$p = 0.418$
HbA1c (%)	5.86 ± 0.50	6.17 ± 0.74	6.58 ± 1.19	$p = 0.138$
Hemoglobin (g/dL)*&	13.17 ± 1.39	11.72 ± 2.19	10.95 ± 1.21	$p < 0.05$*
Indoxyl sulfate (μM)&^	6.54 ± 3.45	41.97 ± 43.96	227.29 ± 92.65	$p \leq 0.05$*
TIrisin (pg/ml)	115.12 ± 108.73	73.13 ± 37.71	72.71 ± 59.61	$p = 0.106$
Myostatin (ng/ml)	2.64 ± 4.49	1.28 ± 0.84	1.29 ± 1.98	$p = 0.188$
Interleukin 6 (pg/ml)	3.67 ± 7.09	2.99 ± 5.09	4.68 ± 4.67	$p = 0.232$

*Control vs. CKD; &Control vs. ESRD; ^CKD vs. ESRD.

of all participants was higher than 1.2 as the suggestion of the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (20).

All participants received a prestudy medical workup, including a physical evaluation, electrocardiography, resting echocardiography, and blood biochemical tests. After enrollment, the participants received assessments of their clinical parameters, baseline hematological and biochemical parameters, baseline DEXA measurements, and myokine concentrations.

2.2. Demographic data and biochemical results

Demographic data were obtained from the medical records at Cardinal Tien Hospital. The diagnoses of congestive heart failure, diabetes mellitus, and hypertension were verified through medical records. Body weight and height were measured after hemodialysis to obtain body mass index. The following predialytic hematological and biochemical parameters were collected from

TABLE 4 The concentration of myokine between the participants with and without lower handgrip strength.

Groups	With lower handgrip strength (n = 22)	Without lower handgrip strength (n = 52)	P-value
Female (percentage)	8 (36.3)	22 (42.2)	
Indoxyl sulfate (μM)	172.06 ± 114.33	97.69 ± 121.93	$P < 0.05$
Irisin (pg/ml)	68.86 ± 44.21	101.95 ± 88.77	$P < 0.95$
Myostatin (ng/ml)	1.28 ± 1.54	2.26 ± 3.56	$P = 0.145$
Interleukin 6 (pg/ml)	3.86 ± 4.89	4.33 ± 6.49	$P = 0.302$

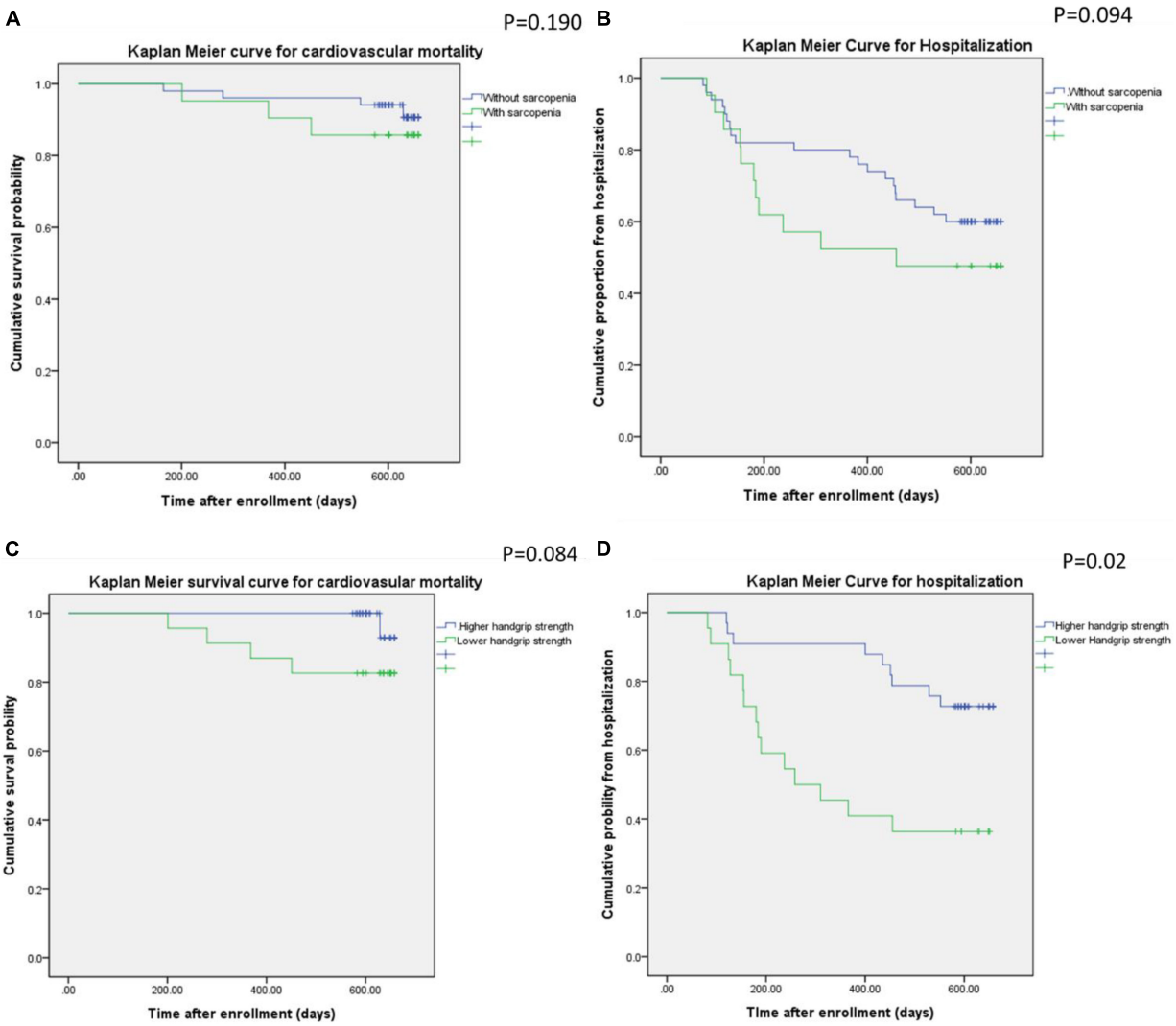

FIGURE 1
Kaplan–Meier curves for cardiovascular mortality and hospitalization in the participants with or without sarcopenia **(A,B)** and low handgrip strength **(C,B)**.

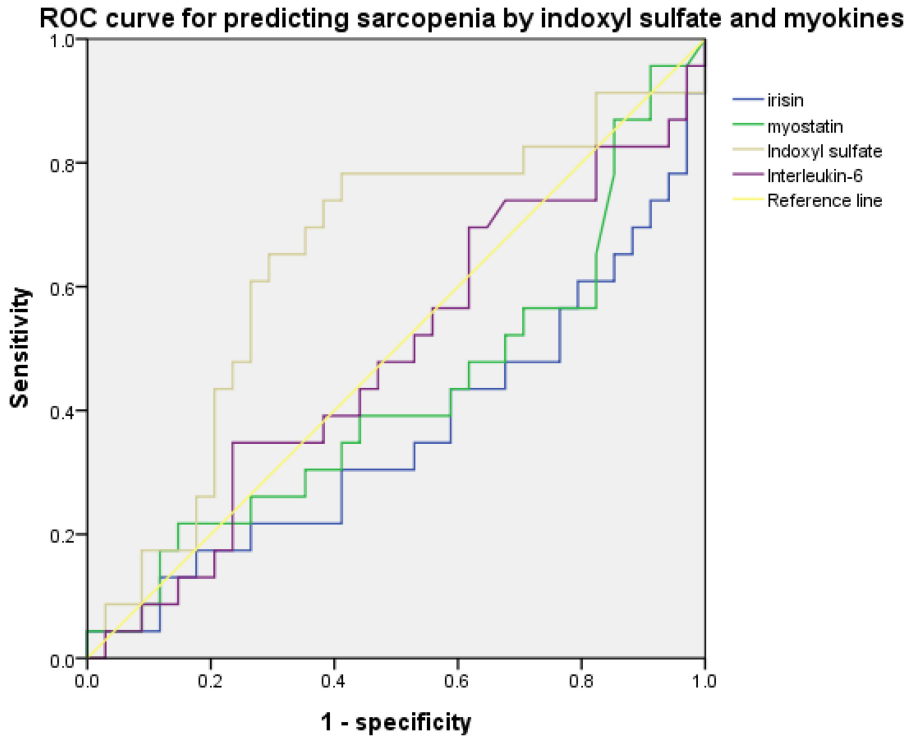

FIGURE 2
ROC curve for predicting sarcopenia based on concentrations of indoxyl sulfate, irisin, myostatin, and Interleukin-6 (indoxyl sulfate: AUC: 0.642, 95% CI: 0.489–0.795, $p = 0.071$; irisin: AUC: 0.361, 95% CI: 0.208–0.513, $p = 0.076$; myostatin: AUC: 0.426, 95% CI: 0.269–0.0.583, $p = 0.345$; interleukin-6: AUC: 0.578, 95% CI: 0.429–0.727, $p = 0.317$).

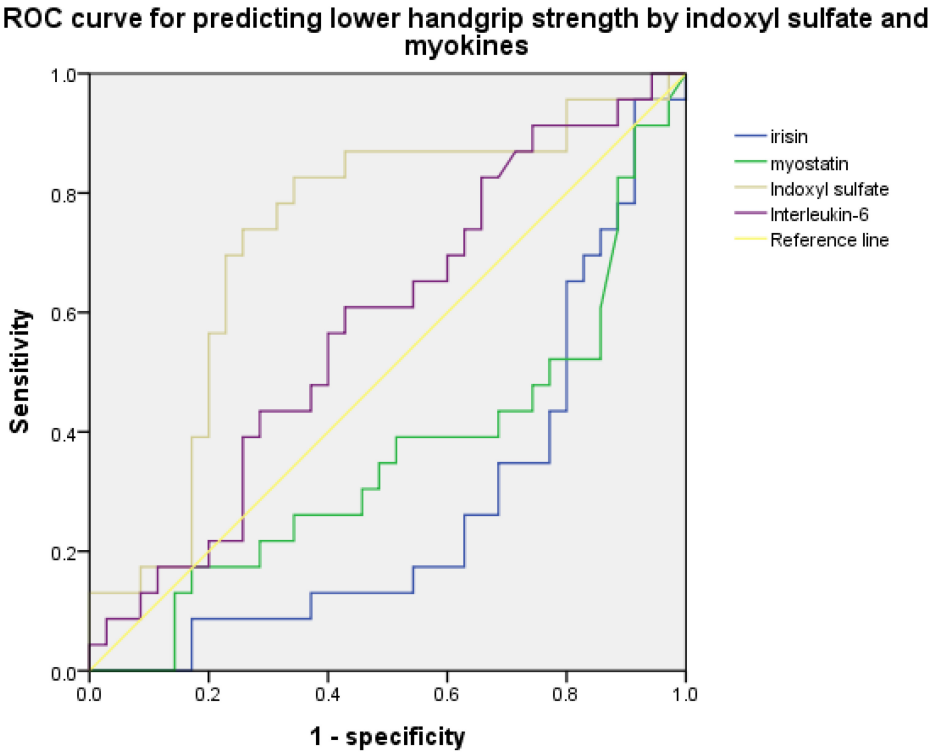

FIGURE 3
ROC curve for predicting low handgrip strength based on concentrations of indoxyl sulfate, irisin, myostatin, and interleukin-6 (indoxyl sulfate: AUC: 0.724, 95% CI: 0.585–0.863, $p < 0.05$; irisin: AUC: 0.276, 95% CI: 0.142–0.410, $p < 0.05$; myostatin: AUC: 0.358, 95% CI: 0.208–0.508, $p < 0.05$; interleukin-6: AUC: 0.578, 95% CI: 0.429–0.727, $p = 0.317$).

each patient within 1 month after obtaining written informed consent: hemoglobin, platelet count, white blood cell count, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, albumin, blood sugar, uric acid, total cholesterol, triglycerides, sodium, potassium, calcium, phosphorus, and intact parathyroid hormone. The eGFR was determined by using the Modification of Diet in Renal Disease Study equation (21).

2.3. Measurement of myokines and indoxyl sulfate

Myokines, including irisin, myostatin, and interleukin-6, were measured by enzyme immunoassay kit (Abbkine, Wuhan, China). Serum samples were drawn to measure biochemical and hematological parameters. Serum was collected under fasting conditions and stored at −80°C for later measurement. The parameters were measured according to the manufacturer's instructions (the inter- and intra-assay coefficients of variability for irisin, myostatin, and interleukin-6 were < 11% and < 9%, respectively). Indoxyl sulfate was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Leadgene Biomedical, Tainan, Taiwan), validated through high-performance liquid chromatography–mass spectrometry (US patent: US10723791B2). The monoclonal antibody against antigenic indoxyl sulfate is generated from 8 to 10 week-old female BALB/c mice with removal rate 97.78% of human plasma. The binding activity, against indole, L-tryptophan or 3-indoleacetic acid (defined by mean absorbance of compounds–spiked wells)/[mean absorbance of blank control wells (B/Bo)] was less than 30%. Briefly, the serum was diluted by 20-fold to a final volume of 100 μL and then added to an equal volume of diluted detection antibody. After 1 h, the ELISA wells were washed, and 3,3',5,5'-tetramethylbenzidine was used for color development. The indoxyl sulfate level was determined on the basis of a standard curve.

2.4. DEXA: measurement of muscle mass

In addition to blood sampling, DEXA imaging was also performed. Patients with ESRD received DEXA scans on the day after hemodialysis. The DEXA imaging process was as follows. The patients were asked to fast overnight and refrain from drinking alcohol for > 8 h before the DEXA scan. During the examination, the patients were asked to wear cotton clothing and remove all metal objects from their persons. Scans were performed using a GE Lunar iDXA (GE Healthcare, Chicago, IL, USA) operated in whole-body scan mode, and the scan was performed in the order of head, upper limbs, lower limbs, and trunk. The whole-body scan of each patient required approximately 20 min to complete.

The appendicular skeletal mass index was defined as the sum of the lean muscle mass of all four limbs divided by the patient's height (in meters) squared (appendicular skeletal muscle/height^2) (22). The relative fat mass indices for the trunk, leg, arm, android, gynoid, and total body fat were obtained according to the method described by Stults-Kolehmainen et al. (23). The scanner manufacturer defined the trunk, leg, android, and gynoid regions as follows: (1) The trunk region comprises the neck, chest, abdominal, and pelvic areas. The upper and lower perimeters of the trunk are the interior edge of the chin and the middle of the femoral necks without touching the brim of the pelvis, respectively. (2) The leg region comprises the pelvic region at an angle perpendicular to the femoral neck. (3) The android region comprises the area between the ribs and the top of the pelvis that is totally enclosed by the trunk region. The upper boundary is 20% of the distance between the iliac crest and the neck, and the lower boundary is at the top of the pelvis. (4) The gynoid region comprises the hips and upper thighs overlapping both the leg and trunk regions (24). The results of these scans were analyzed using the DEXA scanner's integrated software (v12.10.017, GE Healthcare, Chicago, IL, USA).

2.5. Diagnoses of sarcopenia and low handgrip strength

The Asian Working Group for Sarcopenia (AWGS) (25) defines sarcopenia as low muscle mass and low muscle strength. We used the AWGS algorithm to identify sarcopenia as follows: appendicular skeletal muscle index (appendicular skeletal muscle/height^2) of < 7.0 kg/m^2 in men and < 5.4 kg/m^2 in women. Low handgrip strength was defined a handgrip strength of < 28 kg in men and < 18 kg in women (25).

TABLE 5 Univariate and Multivariate logistic regression analyses for the factors associated with low handgrip strength.

	Univariate odd ratio (95% CI)	*p*-value	Multivariate odd ratio (95% CI)	*p*-value
Age (> 65 year/old)	3.136 (1.221–8.058)	0.005	6.728 (1.418–31.33)	0.016
Without CKD (estimated GFR > 60 ml/min)	0.890 (0.643–1.232)	0.356		
Hyperphosphatemia (> 5.5 mg/L)	0.654 (0.35–1.22)	0.171		
Albumin (> 3.5 g/dL)	0.563 (0.29–1.90)	0.231		
Hemoglobin (< 12 g/dL)	0.593 (0.299–1.173)	0.097		
Irisin (> 63 pg/ml)	0.462 (0.235–0.911)	0.019		
Indoxyl sulfate (> 135 μM)	3.485 (1.372–8.852)	0.002	8.525 (1.807–40.207)	0.007
DM	0.725 (0.424–1.344)	0.25		
Hypertension	0.744 (0.546–1.098)	0.135		
Congestive heart failure	1.494 (1.058–2.109)	0.009		

2.6. Cardiovascular mortality and hospitalization assessment

Cardiovascular mortality and hospitalization records were made prospectively by examining all patients who had been enrolled in the study for at least 3 months between 1 April 2018 and 31 December 2021. Each medical chart was reviewed, and a physician assigned the cause of death on the basis of all clinical information available from the Cardinal Tien Hospital emergency department or intensive care unit. Patients who were lost to follow-up after study completion were excluded from this analysis. Cardiovascular mortality was defined as any death directly related to cardiovascular system dysfunction occurring at Cardinal Tien Hospital (including stroke, myocardial infarction, congestive heart failure, or sudden death). The hospitalization assessment comprised all hospital stays lasting at least 1 night that occurred during the 2-year period after diagnosis. These data were collected from hospital admissions records and discharge letters extracted from the general practice records.

2.7. Statistics

Continuous variables are presented as mean ± standard deviation. Categorical values are expressed as percentages. A one-way analysis of variance was used to compare the differences in variables within the three patient groups. We used Pearson's correlation coefficient to assess the predictive performance of individual parameters for low handgrip strength, including advanced age, diabetes mellitus, coronary artery disease, congestive heart failure, and indoxyl sulfate and myokine concentrations. Receiver operating characteristic (ROC) curves were plotted, and the area under the curve (AUC) was estimated. We compared the Kaplan–Meier estimates for 2-year cardiovascular mortality and hospitalization between the groups with and without sarcopenia and between the groups with and without low handgrip strength. All statistical analyses were performed using the statistical package SPSS for Windows (v.17; SPSS, Chicago, IL, USA). A two-tailed p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Low handgrip strength was more prevalent than sarcopenia in patients with CKD and ESRD

Table 1 reveals the demographic characteristics of the three study groups. The numbers of participants in the control, CKD, and ESRD groups were 16, 17, and 42, respectively. The mean age of the participants in the control group (48.5 ± 10.94 years) was lower than that of the participants in the CKD and ESRD groups (65.64 ± 5.93 and 60.68 ± 14.81 years, respectively; $p < 0.05$). The rates of hypertension (18.75, 82.35, and 85.71%; $p < 0.05$) and diabetes mellitus (6.25, 64.7, and 64.9%, $p < 0.05$) were lower in the control group than in the CKD and ESRD groups. The percentage of female

TABLE 6 The concentration of myokine and handgrip strength between the participants based on the quartile divided by age.

Groups	1st quartile based on age (30–46 years old)	2nd quartile based on age (46–61 years old)	3rd quartile based on age (61–69 years old)	4th quartile based on age (69–85 years old)	*P*-value
Handgrip strength (kg)*	31.79 ± 8.64	25.96 ± 8.28	26.83 ± 11.19	19.23 ± 9.44	$P < 0.05$
Irisin (pg/ml)*	122.39 ± 124.96	85.14 ± 61.88	61.66 ± 27.93	63.33 ± 21.85	$P < 0.05$
Myostatin (ng/ml)	2.98 ± 5.07	1.59 ± 1.71	0.96 ± 0.47	1.05 ± 0.53	$P = 0.087$
Interleukin 6 (pg/ml)	3.40 ± 3.00	4.66 ± 6.05	3.54 ± 4.07	4.44 ± 7.19	$P = 0.634$

*1st quartile vs. 4th quartile.

TABLE 7 Dual-energy X-ray absorptiometry parameters by group.

	Control	CKD	ESRD	*p*-value
Android fat mass (kg)	2.16 ± 0.87	2.11 ± 0.95	2.09 ± 0.80	0.969
Android lean mass (kg)	3.64 ± 0.67	3.79 ± 1.06	3.63 ± 0.86	0.814
Android total mass (kg)	5.08 ± 1.38	5.97 ± 1.71	5.73 ± 1.52	0.870
Android fat percentage (%)	36.34 ± 7.08	35.45 ± 9.01	36.04 ± 6.45	0.944
Gynoid fat mass (kg)	3.50 ± 1.32	2.82 ± 1.00	2.75 ± 0.85	0.081
Gynoid lean mass (kg)	7.26 ± 1.20	7.03 ± 1.95	6.51 ± 1.25	0.242
Gynoid total mass (kg)	10.85 ± 1.81	9.89 ± 2.42	9.26 ± 1.73	0.059
Gynoid fat percentage (%)	32.00 ± 8.78	28.40 ± 7.79	29.60 ± 6.05	0.407
Android/gynoid fat ratio	1.11 ± 0.31	1.40 ± 0.30	1.21 ± 0.18	<0.05
The ratio of trunk/leg fat mass	1.12 ± 0.26	1.40 ± 0.47	1.28 ± 0.20	0.05
The ratio of total trunk/limb mass	1.22 ± 0.29	1.55 ± 0.46	1.56 ± 0.26	<0.05
Lean mass/height^2	16.64 ± 2.65	16.78 ± 1.60	16.86 ± 2.24	0.949
Appendicular skeletal muscle/height^2	6.89 ± 1.32	7.14 ± 0.90	6.82 ± 1.20	0.641

was higher in the control group (75, vs. 23.5% for CKD and 34.1% for ESRD group). The mean duration of the ESRD subjects receiving maintenance renal replacement therapy was 2.23 ± 1.45 years (not demonstrated in Table 1).

Table 2 reports the prevalence of low handgrip strength and sarcopenia in the different groups. Low handgrip strength was more prevalent in the ESRD group than in the control and CKD groups (41.46 vs. 25% and 5.85%, respectively; $p < 0.05$). The prevalence of sarcopenia was similar among the groups (12.5, 17.6, and 19.5% for the control, CKD, and ESRD groups, respectively; $p = 0.864$). The body mass index was similar between groups ($p = 0.398$).

Table 3 displays the results of hematological and biochemical analyses. The ESRD group exhibited significant baseline differences in blood urea nitrogen, creatinine, and eGFR when compared with the CKD and control groups. Moreover, the concentrations of sodium, albumin, and hemoglobin were significantly lower in the ESRD group than in the other two groups [sodium: 138.10 ± 2.54, 140.13 ± 2.39, and 140.73 ± 3.80 mEq/L ($p < 0.05$); albumin: 4.03 ± 0.34, 4.33 ± 0.42, and 4.23 ± 0.32 g/dL ($p < 0.05$); and hemoglobin: 10.95 ± 1.21, 13.17 ± 1.39, and 11.72 ± 2.19 g/dL, ($p < 0.05$) in the ESRD, control, and CKD groups, respectively]. Conversely, the concentrations of phosphorus was significantly higher in the ESRD group than in the other two groups [phosphorus: 5.44 ± 1.81, 3.81 ± 0.51, and 4.22 ± 0.72 mg/dL ($p < 0.05$); intact parathyroid hormone: 345.88 ± 291.67, 53.75 ± 25.82, and 157.43 ± 162.71 pg/ml ($p < 0.05$) in the ESRD, control, and CKD groups, respectively]. Serum indoxyl sulfate was significantly lower in the control group than in the other two groups (6.54 ± 3.45, 41.97 ± 43.96, and 227.29 ± 92.65 μM in the control, CKD, and ESRD groups, respectively; $p < 0.05$). Irisin was higher in the control group than in the other two groups, but the difference was non-significant. The concentrations of myostatin and interleukin-6 were similar among the groups.

Table 4 illustrated the concentration of myokine and indoxyl sulfate between the participants with and without lower handgrip strength. The indoxyl sulfate was higher in the lower handgrip strength group (172.06 ± 114.33 μM vs. 97.69 ± 121.93 μM, $p < 0.05$). The irisin concentration was lower in the lower handgrip strength group (68.86 ± 44.21 pg/ml, vs. 101.95 ± 88.77 pg/ml, $p < 0.05$).

3.2. Low handgrip strength—but not sarcopenia—was associated with hospitalization in all participants

Figure 1 illustrates the Kaplan–Meier plot for cardiovascular mortality and hospitalization for all participants during the 2-year follow-up period, with stratification for sarcopenia and low handgrip strength. The differences in cardiovascular mortality and hospitalization rates between the participants with and without sarcopenia were non-significant ($p = 0.191$ for cardiovascular mortality; $p = 0.094$ for hospitalization). Conversely, the participants with low handgrip strength experienced significantly more hospitalizations ($p = 0.02$) during the 2-year follow-up period than the patients with normal handgrip strength did. Nevertheless, cardiovascular mortality rates were similar in the participants with and without low handgrip strength ($p = 0.084$).

3.3. Indoxyl sulfate was associated with low handgrip strength and clinical outcomes

An ROC curve for predicting sarcopenia or low handgrip strength was used to investigate the relationship between myokine and indoxyl sulfate concentrations. Figures 2, 3 present the ROC curves for predicting sarcopenia and low handgrip strength based on myokine and indoxyl sulfate in the total population. Figure 2 illustrates the ROC curve for predicting sarcopenia. On the basis of this model, the concentrations of indoxyl sulfate, irisin, myostatin, and interleukin-6 were not predictive of sarcopenia (indoxyl sulfate: AUC: 0.642, 95% CI: 0.489–0.795, $p = 0.071$; irisin: AUC: 0.361, 95% CI: 0.208–0.513, $p = 0.076$; myostatin: AUC: 0.426, 95% CI: 0.269–0.0.583, $p = 0.345$; interleukin-6: AUC: 0.578, 95% CI: 0.429–0.727, $p = 0.317$). Figure 3 illustrates the ROC curve for predicting low handgrip strength. The concentrations of indoxyl sulfate and irisin were predictive of low handgrip strength in the total population (indoxyl sulfate: AUC: 0.724, 95% CI: 0.585–0.863, $p < 0.05$; irisin: AUC: 0.276, 95% CI: 0.142–0.410, $p < 0.05$). The concentrations of myostatin and interleukin-6 were not predictive of low handgrip strength (myostatin: AUC: 0.358, 95% CI: 0.208–0.508, $p < 0.05$; interleukin-6: AUC: 0.578, 95% CI: 0.429–0.727, $p = 0.317$). The cutoff values for irisin and indoxyl sulfate for diagnosing low handgrip strength were 63 pg/ml and 136 μM, respectively.

TABLE 8 Correlations between indoxyl sulfate and body composition in total population.

	Indoxyl sulfate	*p*-value
Android fat mass (kg)	−0.029	0.823
Android lean mass (kg)	−0.004	0.974
Android total mass (kg)	−0.025	0.846
Android fat percentage (%)	−0.24	0.852
Gynoid fat mass (kg)	−0.248	0.052
Gynoid lean mass (kg)	−0.227	0.076
Gynoid total mass (kg)	−0.304	0.016
Gynoid fat percentage (%)	−0.092	0.47
Android/gynoid fat ratio	0.024	0.849
The ratio of trunk/leg fat mass	0.077	0.586
The ratio of total trunk/limb mass	0.211	0.089
Lean mass/height^2	0.183	0.141
Appendicular skeletal muscle/height^2	0.19	0.133
Fat mass/height^2	−0.215	0.083

TABLE 9 Comparison of gynoid total mass, lean mass, and fat mass in participants with and without low handgrip strength.

	With low handgrip strength	Without low handgrip strength	*p*-value
Gynoid fat mass (kg)	2.61 ± 0.63	3.08 ± 1.22	0.097
Gynoid lean mass (kg)	6.40 ± 1.38	7.30 ± 1.40	0.039
Gynoid total mass (kg)	9.02 ± 1.68	10.42 ± 1.97	0.017
Gynoid fat percentage (%)	29.20 ± 5.71	29.14 ± 8.65	0.98

Table 5 illustrates the univariate and multivariate analyses of the factors associated with low handgrip strength. A univariate analysis was performed to investigate the odds ratios (ORs) for low handgrip strength among groups when stratified by demographic, hematological, and biochemical parameters (demographic: age; history of diabetes mellitus, congestive heart failure and hypertension; biochemical and hematological parameters: serum albumin > 3.5 g/dL, phosphorus > 5.5 mg/dL, hemoglobin > 12 g/dL). Irisin concentrations were also included in this analysis due to the association with low handgrip strength. In the univariate analysis, older age (> 65 years), higher indoxyl sulfate concentrations (> 136 μM), and history of congestive heart failure were associated with low handgrip strength [OR for indoxyl sulfate: 3.485, 95% confidence interval (CI): 1.372–8.852, $p = 0.02$; OR for age > 65 years: 3.136, 95% CI: 1.221–8.058, $p = 0.005$; OR for congestive heart failure: 1.494, 95% CI: 1.058–2.109, $p = 0.009$]. Higher irisin levels (> 63 pg/ml) were negatively correlated with low handgrip strength (OR: 0.462, 95% CI: 0.235–0.911, $p = 0.019$). In the multivariate regression analysis with age, irisin, indoxyl sulfate, congestive heart failure, higher indoxyl sulfate concentrations, and advanced age were associated with the risk of low handgrip strength (OR for age: 6.728, 95% CI: 1.418–31.33, $p = 0.016$; OR for indoxyl sulfate: 8.525, 95% CI: 1.807–40.207, $p = 0.007$). To evaluate the effect of age on the expression of myokine and handgrip strength, we divided the subjects into quartiles based on the age concentration (Table 6). The handgrip strength was the highest in the 1st quartile,(31.79 ± 8.64 kg, vs. 4th quartile with 19.23 ± 9.44 kg, $p < 0.05$) The irisin was also the highest in the 1st quartile (122.39 ± 124.96 pg/ml, vs. 4th quartile with 63.33 ± 21.85 pg/ml, $p < 0.05$).

Figure 4 illustrated the Kaplan–Meier curve for cardiovascular mortality and the hospitalization based on the higher or lower concentration of indoxyl sulfate. The concentration of participants with higher indoxyl sulfate concentration (> 63 pg/ml) was associated with higher incidence of cardiovascular mortality ($p = 0.03$) and higher hospitalization rate ($p = 0.008$) under duration of 600 days.

3.4. Indoxyl sulfate was associated with decreased gynoid total mass

Table 7 displays the DEXA parameters of the participants in all three groups. The appendicular skeletal muscle indices were similar among the groups (6.89 ± 1.32, 7.14 ± 0.90, and 6.82 ± 1.20 kg/m^2 for the control, CKD, and ESRD groups, respectively; $p = 0.641$). The control group had the lowest android to gynoid and trunk to limb fat mass ratios among the three groups (android/gynoid: 1.11 ± 0.31, 1.40 ± 0.30, 1.21 ± 0.18, $p < 0.05$; trunk/limb: 1.22 ± 0.29, 1.55 ± 0.46, 1.56 ± 0.26, $p < 0.05$ for the control, CKD, and ESRD groups, respectively). Table 8 reveals the correlations between DEXA parameters and indoxyl sulfate concentrations in the total population. The gynoid total mass was negatively correlated with indoxyl sulfate concentration ($r = -0.304$, $p = 0.016$). Table 9 reveals the difference in gynoid parameters between patients with and without low handgrip strength in the total population. The total and lean gynoid mass were lower in participants with low handgrip strength. Table 10 illustrated the correlation between indoxyl sulfate concentration with other hematologic or biochemical parameters with difference between groups in Table 3. The concentration of indoxyl sulfate was positive correlated with the concentration with phosphorus ($r = 0.363$, $p < 0.05$). There was no other correlation between indoxyl sulfate and other parameters.

TABLE 10 The correlation between the concentration of indoxyl sulfate and age, hematologic or biochemical parameters with difference between groups in Table 3.

	Correlation coefficient	*p*-value
Age	−0.179	0.263
Sodium	−0.260	0.101
Phosphorus	0.363	0.018
Albumin	0.001	0.996
Hemoglobin	−0.130	0.419

4. Discussion

In our study, low handgrip strength was common among patients with CKD and ESRD. We observed a correlation between low handgrip strength and high hospitalization rates, but no such association existed between sarcopenia and hospitalization. Indoxyl sulfate concentration was an important factor contributing to low handgrip strength, and the multivariate logistic regression analysis revealed that indoxyl sulfate counteracted the protective effect of irisin. Furthermore, the indoxyl sulfate concentration was inversely correlated with gynoid total mass.

The DEXA scan utilized two low-dose X-ray beams of different levels to detect fat, bone mass, and muscle mass. The results revealed decreased appendicular skeletal muscle mass relative to height (kg/m^2), and the percentages of appendicular skeletal muscle wasting were similar among the groups. Two diagnostic criteria are used for identifying sarcopenia in patients receiving peritoneal dialysis or hemodialysis: (1) an appendicular skeletal muscle index more than two standard deviations below the reference index for healthy young adults (26–28) and (2) an appendicular lean mass index at least 20 percentiles below the general population (28). The incidence of sarcopenia varies from 20 to 73.5% depending on the diagnostic criteria. DEXA serves as an important tool for diagnosing sarcopenia, but several factors can impair the diagnostic efficacy in patients with CKD. Nevertheless, variations in fat, bone mass, and muscle mass were noted in the DEXA results. In non-dialysis CKD, visceral adipose tissue is highly associated with obesity, and adipose tissue is associated with metabolic syndrome or cardiovascular complications such as coronary artery calcification (29, 30). In patients undergoing dialysis, hydration status and the modality of renal replacement therapy influence the results of body composition scans. In patients receiving peritoneal dialysis, the amount of peritoneal dialysate might influence the patient's hydration status and result in an overestimation of muscle mass (31). Therefore, sarcopenia diagnoses can be inconsistent across study groups. Our study demonstrated that low handgrip strength was a more reliable predictor of hospitalization and poor clinical outcomes during the 2-year follow-up than radiologically diagnosed sarcopenia, as Figure 1 illustrated. Low handgrip strength was associated with higher mortality and the development of chronic illnesses such as malignant cancer among patients in all age groups (32–34).

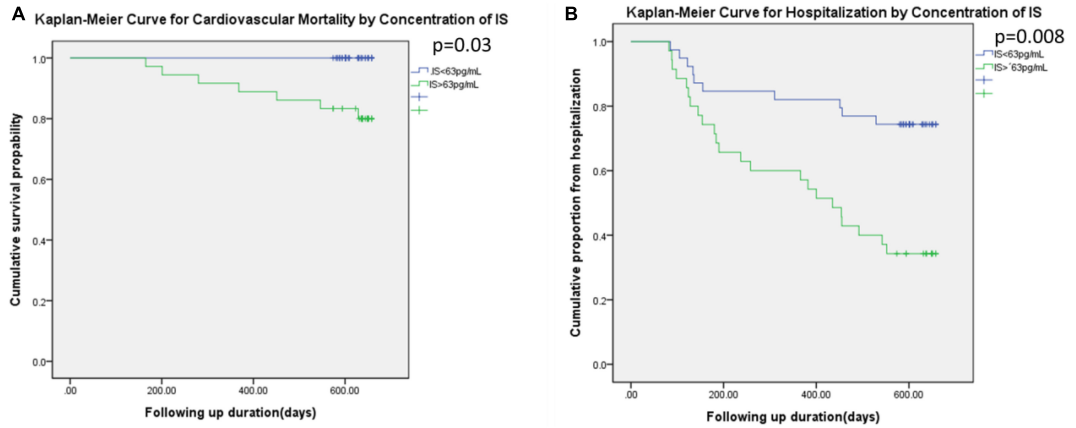

FIGURE 4
Kaplan–Meier curve for the cardiovascular mortality **(A)** and the hospitalization **(B)** based on the IS level (higher than 63 pg/ml or not).

Although the ability of handgrip strength to represent overall muscle strength has not been determined, handgrip strength can serve as an indicator of cardiovascular fitness (35–36). In CKD patients, low handgrip strength was associated with comorbidities such as hypernatremia (19) and poor renal outcomes (37). Besides, several comorbidities common in CKD/ESRD could influence the handgrip strength, such as anemia (38, 39). The accumulation of uremic toxin, such as indoxyl sulfate, could also influence overall fitness vial influencing ventricular remodeling or impairment of immunity (40, 41). Therefore the concentration of indoxyl sulfate also reflected the 2-year cardiovascular mortality and the hospitalization in our study (**Figure 4**). The overall fitness, indicated by lower handgrip strength, was associated with the higher hospitalization by infection as previous reports (42, 43). Therefore, the overall decreased fitness, rather than the severe decrease in skeletal mass by DEXA parameters, was more associated the clinical outcome in vulnerable subjects, such as CKD patients.

Indoxyl sulfate is a protein-bound uremic toxin derived from tryptophan. After tryptophan is ingested, it is transformed into indole derivatives and indoxyl sulfate by the hepatic cytochrome P450 2E1. The organic anion transporter (OAT) within the renal proximal tubules normally governs the excretion of indoxyl sulfate, but in patients with CKD, tubulointerstitial fibrosis decreases OAT function and leads to increased plasma concentrations of indoxyl sulfate (16, 4). Indoxyl sulfate causes cellular damage either through direct generation of oxidative stress or by modulating transcription factors such as aryl hydrocarbon receptors (44). In patients receiving maintenance hemodialysis or peritoneal dialysis (45, 46), indoxyl sulfate could not be removed effectively, and the toxic effect of indoxyl sulfate on skeletal muscle has been demonstrated *in vivo* and *in vitro*. As the **Figure 4** illustrated, the higher concentration of indoxyl sulfate was associated higher cardiovascular mortality and hospitalization in total population. Based on the previous study, the indoxyl sulfate might influence the handgrip strength vial: (1) the decrease ATP generation in mitochondria by inducing the endoplasmic reticulum stress in skeletal by dysregulating tricarboxylic acid cycle (17); (2) inducing the myogenesis by arousing the unfolded protein response in endoplasmic reticulum (6); (3) influencing the overall fitness vial the decrease cardiac function, impairment of the hematopoiesis, or angiogenesis of peripheral arterys (47, 40, 48). We noticed that the indoxyl sulfate concentration was negatively associated with total gynoid mass. Additionally, low handgrip strength was correlated with low gynoid total and lean mass. This finding corresponds to the findings of Chao et al. who reported that lower total body mass and lean mass were associated with frailty in patients undergoing dialysis. Chao et al. also noted that appendix fat, in contrast with trunk fat, was higher in patients with frailty (49). A decrease in weight-bearing capacity might further impair the cardiovascular fitness of patients with CKD and ESRD (50). In light of our results, we believe that protein-bound uremic toxins such as indoxyl sulfate might influence the weight-bearing lean skeletal muscle mass of the gynoid area. However, further research into the pathophysiological mechanisms is needed to verify this theory.

Irisin is the myokine released from skeletal muscle during exercise. Irisin expression is regulated by the peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1α (PGC-1α) and fibronectin type III domain containing 5 (FNDC5) axis (51). After being released from skeletal muscle, irisin modulates the energy expenditure in adipose tissue by stimulating the expression of brown preadipose genes in beige precursor fat cells (52). Additionally, irisin could activate osteoblast differentiation (53) or inhibit the apoptosis of osteoblasts by reducing the generation of inflammasomes (54). Irisin also maintains osteocytic survival by binding to the αV class of integrins (55). Furthermore, irisin could modulate the expression of mitochondrial uncoupling protein 2 to reduce oxidative stress after ischemia or reperfusion injury. Because obesity and metabolic syndrome develop before ESRD, irisin could counteract the hazard of obesity and therefore provide renoprotection (56). Serum concentrations of irisin decrease in patients with CKD. Wen et al. demonstrated that serum irisin concentration was negatively associated with the serum concentrations of blood urea nitrogen and creatinine (57, 58). A lower concentration of irisin was associated with cardiovascular mortality in patients with CKD (59). Our study is the first to demonstrate that irisin concentration is negatively correlated with lower muscle strength in patients with CKD without influence on lean muscle mass composition. As mentioned, protein-bound uremic toxins such as indoxyl sulfate might hamper mitochondrial ATP generation, counteracting the protective effect of irisin in several aspects. Patients with CKD have several risk factors that dysregulate the PGC-1α–FNDC5 axis, such as vitamin D deficiency and the accumulation of protein-bound uremic toxins (60, 61). An *in vitro* study demonstrated that indoxyl sulfate dysregulated

the expression of PPARγ in C2C12 cells and therefore increased autophagy levels (62). On the basis of this evidence, we believe that indoxyl sulfate might counteract the effect of irisin and contributed to the lower muscle strength observed in patients with CKD and ESRD. Nevertheless, further investigation is needed to clarify the detailed mechanisms of irisin expression and to explore possible interventions for patients with low handgrips, such as vitamin D supplements or exercise (63, 64). In contrast to the situation regarding irisin, we observed no relationship between myostatin alone and low handgrip strength in this cohort study, and the concentration of myostatin was even higher in control group (without statistic difference). Myostatin, as part of the TGF-beta superfamily, is secreted from skeletal muscle and serves as the negative regulator of myocytes. The active form of myostatin inhibits the phosphoinositide 3-kinase–protein kinase B signaling pathway of skeletal muscle (65) and induces apoptosis of myocytes through regulation of gene-expressed autophagy. Notably, apoptosis and myostatin mRNA are upregulated in the skeletal muscle of patients with CKD (66). A possible mechanism is that the percentage of congestive heart failure is higher in CKD and ESRD group. It has been noticed that the myostatin serves as the inhibitor to alleviate the development of the cardiac fibrosis, and the origin of myostatin might be derived from the cardiac and adipose tissue (67, 68). Therefore, the influence of myostatin on sarcopenia was equivocal in the patients with CKD and ESRD (69, 70). We also observed that the concentration of interleukin-6 was lower in the control group without statistic difference. Interleukin-6 has been linked to lower handgrip strength in other studies because the concentration reflected the inflammatory status along with the aging process (71, 72). The mean value was higher in the subjects with higher handgrip strength without statistic difference. A possible explanation was the difference in gender. Miko et al. demonstrated that the higher plasma IL-6 concentration in male gender was associated with better skeletal muscle condition (73). The female percentage was higher in the control group, and therefore the IL-6 concentration might be lower in the control group. The interactions of anabolic or catabolic myokines with the regulators for myokines in patients with CKD warrant further advanced study. On the basis of our results, we believe the protective effect of irisin might be mitigated by factors other than myokines.

Hypoalbuminemia has been noticed as a possible factor contributing to lower handgrip strength in our study. Hypoalbuminemia and other indicators for malnutrition are associated with cardiovascular events in CKD patients (74). For the CKD patients with or without dialysis, the dietary restriction of protein and sodium might decrease the adequate calorie uptake and therefore worsen the malnutrition (75, 76). Beyond the insufficient calorie intake, the underlying factors such as insulin resistance, vitamin D deficiency or excessive homocysteine (77, 78) could worsen the inflammatory status in CKD patients and therefore worsen the cardiovascular function. The assessment for screening the malnutrition-inflammation-atherosclerosis syndrome (MIA syndrome), the conventional Subjective Global Assessment or protein energy wasting are important for the routine care in CKD patients (79, 80). Besides, the measurement for inflammatory indicators such as C-reactive protein might be important for caring the CKD patients (81, 82). From our study, the body mass index was similar between groups. Therefore the indicators for inflammation, which were not measured in our study, and its interaction with uremic toxin or myokine might be helpful for providing the possible therapeutic strategies in managing sarcopenia/frailty in CKD patients.

This study has several limitations. First, the study was initiated in the single institute. Second, the influence of age for the lower handgrip strength was noted in our study from the multivariate logistic regression (odd ratio: 6.728, 95% confidence interval: 1.418–31.33). Our data also demonstrated that the irisin concentration was lower in the elder subjects (83). It has been noticed that the age could influence the handgrip strength, although the factors contributing for lower handgrip strength variated in different studies with different designs (84–86). The influence of aging might also be altered by increasing the sample size or altering the enrollment criteria accordingly. Third, the measurement for indoxyl sulfate in the study was the ELISA method. The detection of indoxyl sulfate is mostly by the liquid chromatography - mass spectrometry (LC–MS) and high–performance liquid chromatography (HPLC) (87, 88). Although the validation has been validated, to measure the concentration of indoxyl sulfate with LC-MS or HPLC might provide more direct evidence between the protein bounded uremic toxin and clinical events. Fourth, according to the literature, handgrip strength, in contrast with lean muscle mass, predicts clinical outcomes. Our study revealed a cross-sectional, but not longitudinal, correlation between uremic toxins and myokines. A longitudinal follow-up might provide a better understanding of the variation in loss of handgrip strength. On the basis of other studies, we speculate that the administration of recombinant irisin might improve the muscle expression of irisin and therefore influence skeletal muscle mass (89). However, we still lack a specific therapeutic strategy for maintaining grip strength as opposed to skeletal muscle mass. Further therapeutic strategies are necessary for promoting the maintenance of muscle strength; therefore, longitudinal studies and *in vivo* studies should be initiated to research the interaction between uremic toxins and the expression of myokines in patients with CKD.

In conclusion, low handgrip strength—but not skeletal muscle mass—was associated with hospitalization in patients with CKD and ESRD. Low handgrip strength was also associated with higher serum concentrations of uremic toxins (namely, indoxyl sulfate) and lower concentrations of irisin compared with the general population.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Cardinal Tien Hospital CTH-107-3-5-027. The patients/participants provided their written informed consent to participate in this study.

Author contributions

K-CL: conceptualization, methodology, and software. S-ML: data curation and writing—original draft preparation. C-MZ and C-LL: visualization and investigation. W-CL and K-CH:

supervision. Y-ML and M-TL: software and validation. Y-CH: writing—reviewing and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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The prevalence of osteoporosis in China, a community based cohort study of osteoporosis

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Background: Osteoporosis has already been a growing health concern worldwide. The influence of living area, lifestyle, socioeconomic, and medical conditions on the occurrence of osteoporosis in the middle-aged and elderly people in China has not been fully addressed.

Methods: The study was a multicenter cross-sectional study on the middle-aged and elderly permanent residents, which gathered information of 22,081 residents from June 2015 to August 2021 in seven representative regions of China. The bone mineral density of lumbar vertebrae and hip were determined using the dual-energy X-ray absorptiometry densitometer instruments. Serum levels of bone metabolism markers were also measured. Information about education, smoking, and chronic diseases were also collected through face-to-face interviews. Age-standardized prevalence and 95% confidence intervals (CIs) of osteopenia and osteoporosis by various criteria were estimated by subgroups and overall based on the data of China 2010 census. The relationships between the osteoporosis or osteopenia and sociodemographic variables or other factors were examined using univariate linear models and multivariable multinomial logit analyses.

Results: After screening, 19,848 participants (90%) were enrolled for the final analysis. The age-standardized prevalence of osteoporosis was estimated to be 33.49% (95% CI, 32.80–34.18%) in the middle-aged and elderly Chinese permanent residents, for men and women was 20.73% (95% CI, 19.58–21.87%) and 38.05% (95% CI, 37.22–38.89%), respectively. The serum concentrations of bone metabolic markers, and calcium and phosphorus metabolism were influenced by age, body mass index (BMI), gender, education level, regions, and bone mass status. Women, aged 60 or above, BMI lower than 18.5 kg/m^2, low education level including middle school, primary school and no formal education as well as current regular smoking, a history of fracture were all significantly associated with a higher risk of osteoporosis and osteopenia in the middle-aged and elderly people.

Conclusions: This study revealed dramatic regional differences in osteoporosis prevalence in China, and female, aged 60 or older, low BMI, low education level, current regular smoking, and a history of fracture were associated with a high risk of osteoporosis. More prevention and treatment resources should be invested into particular population exposed to these risk factors.

KEYWORDS

prevalence, osteoporosis, China, epidemiology, risk factors, cross-sectional study

Introduction

Osteoporosis is a systemic skeletal disease characterized by loss of bone mass and micro architectural integrity that lead to increased bone fragility and risk of fracture (1). The prevalence of osteoporosis has increased over the past years in China (2). According to the recent multicenter survey, the age-standardized prevalence of osteoporosis in China was 6.46 and 29.13% for men and women aged 50 years and older, respectively, and the bone mineral density (BMD) values differed by demographic characteristics (3). Furthermore, ∼2.33 million osteoporotic fractures occurred in 2010 in China, which was estimated to double by 2035 (4).

China is one of the most populous countries in the world with vast territory, and the prevalence and risk factors of osteoporosis varies in different regions of China. With the development of science and technology, people's life quality and health status have been significantly improved in the past decades. However, there are still huge differences in living style, socioeconomic conditions and medical status in different regions of China, such as smoking, education level, economic disparity, and chronic diseases (5–12), which may contribute to the different prevalence of osteoporosis.

To address this issue, we undertook the China Community-based Cohort of Osteoporosis (CCCO) to assess the prevalence of osteoporosis and osteopenia as well as associated risk factors.

Methods and analysis

Recruitment of the participants

The study, which was a multicenter cross-sectional study, was a part of the registered protocol at Clinical trials.gov (NCT02958020). The protocol was approved by the Institutional Review Board at Longhua Hospital affiliated to the Shanghai University of Traditional Chinese Medicine (2016LCSY065), and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All participants provided written informed consent before participation.

We used a multistage stratified cluster random sampling method to enroll a sample of people who would be representative of adults in China (Figure 1). In stage 1, we randomly selected seven provinces

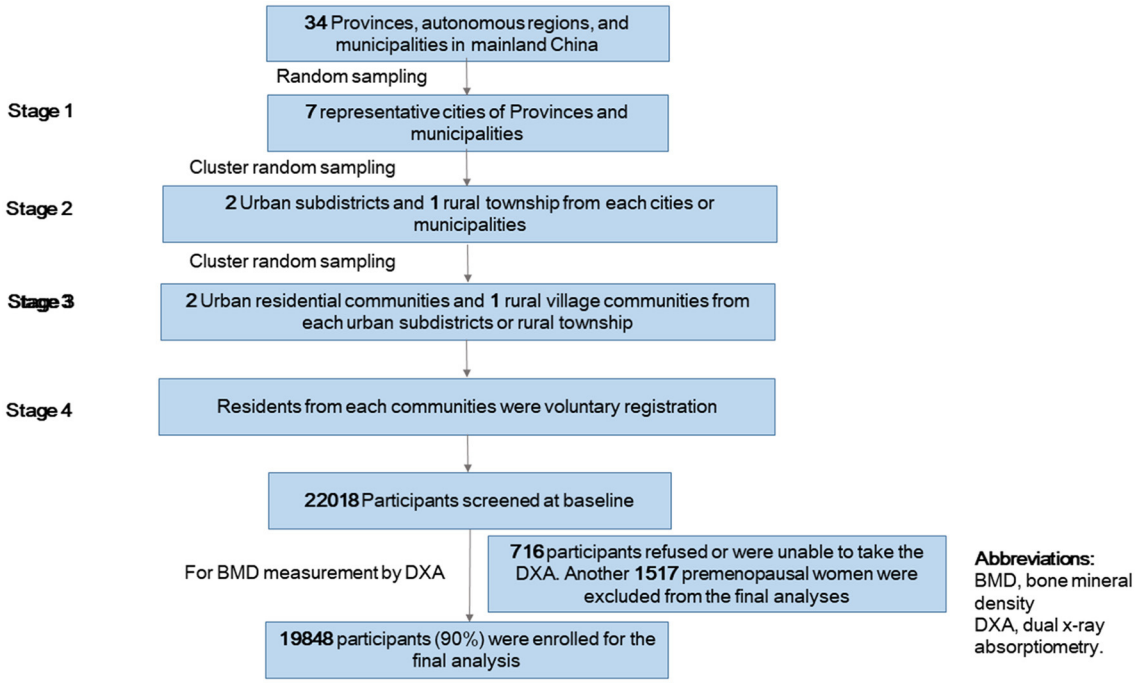

FIGURE 1
Flowchart of participant selection.

in China, including Shanghai (east), Guangdong (south), Gansu (west), Beijing (north), Jilin (north-east), Yunnan (south-west) and Jiangxi (south-west). In stage 2, we used a cluster random sampling method to select 2 urban subdistricts and 1 rural township from each cities or municipalities. In stage 3, we used cluster random sampling method to select 2 urban residential communities and 1 rural village communities from each urban subdistricts or rural township. In stage 4, residents from each community were voluntary registration. Eligible participants were permanent residents including women aged ≥40 years old and men aged ≥50 years old. Lactating or pregnant women and residents with mental health problems, acute infectious diseases and severe physical diseases who could not cooperate with the investigations were excluded. Totally 22,081 residents responded and signed the informed consent forms from June 2015 to August 2021.

Questionnaires and physical examinations

All the participants completed paper-based questionnaires through face-to-face interviews. The questionnaires contained information about age, gender, province, education level, smoking status, history of fracture and chronic diseases including hyperlipidemia, hypertension, and diabetes. Education level was graded into six categories: no formal education, primary school, middle school, high school, college, university, or higher education. Smoking status was graded into four categories: never smoke, former smoker, current regular smoker, or passive smoker. Information about bone fracture history and chronic diseases was obtained from the outpatient and emergency treatment record books provided by the participants.

Physical examinations

Barefoot body weight with indoor clothing and height were measured. Body mass index (BMI) was calculated as weight (kg)/height squared (m^2). BMI was categorized into: underweight, BMI < 18.5 kg/m^2; normal weight, 18.5–24.9 kg/m^2; overweight, ≥25 kg/m^2.

Measurement of serum bone metabolism markers and calcium and phosphorus metabolism indicators

After an overnight fasting, the venous blood samples of all the participants were collected in non-EDTA tubes. Within 2 h after the blood collection, the blood samples were centrifuged at 3,000 rpm for 15 min at room temperature to separate the serum. Serum concentrations of N-terminal propeptide of type I collagen (PINP), β-C-terminal telopeptide of type I collagen (β-CTX), osteocalcin (OST) were measured through an electrochemiluminescence immunoassay. Serum alkaline phosphatase (ALP) concentration was measured through a continuous monitoring technique. Total 25(OH)D [25(OH)D_3 and 25(OH)D_2] was detected using a sensitive and specific high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) method, and parathormone (PTH) was detected through a chemiluminescence immunoassay. The o-cresolphtalein-complexone method and phosphomolybdate ultraviolet colorimetry were applied to detect total calcium (Ca) and phosphorus (P), respectively.

Assessment of BMD and osteoporosis diagnosis

The BMD values of each single lumbar vertebra (L1–L4), the total lumbar vertebra (L1–4) and the total left hip joint (femoral neck, trochanter, and intertrochanteric region) were measured using the dual-energy X-ray absorptiometry densitometer (DXA, Hologic Discovery CI, Bedford, MA, USA) instruments. The instruments used in all the centers were of the same model, and passed the annual verification. Daily calibration program was performed each time the instrument was powered on.

For osteoporosis diagnosis, the BMD values were expressed as *T*-scores (number of standard deviations above/below the mean peak BMD of healthy young-adults of the same race and same gender). According to the Guideline for diagnosis and treatment of primary osteoporosis issued by the Chinese Society of Osteoporosis and Bone Mineral Research in 2017, participants with *T*-scores of any site ≥-1.0 were considered not having osteoporosis or osteopenia. Participants with *T*-scores of more than −2.5, and <-1.0 were diagnosed as having osteopenia, and those with *T*-score ≤ −2.5 as having osteoporosis (13).

Statistical analysis

As first step, the characteristics of the participants were shown as the mean and standard deviation, median and the interquartile range (IQR) or number and proportion in the overall population and in subgroups of gender. The bone metabolic indicators were shown as the mean and standard deviation.

Age-standardized prevalence and 95% confidence intervals (CIs) of osteopenia and osteoporosis by various criteria were estimated by subgroups and overall based on the data of China 2010 census.

The relationships between the osteoporosis or osteopenia and sociodemographic variables or other factors were examined using univariate linear models separately. A multivariable multinomial logit analysis was used to examine the association of variables with the odds of osteoporosis or osteopenia. All *P*-values were 2-tailed and had not been adjusted for multiple testing. All *P*-value < 0.05 was considered statistically significant. All statistical analyses were conducted using the SAS system, version 9.3 (SAS Institute Inc), SUDAAN software, version 10.0 (Research Triangle Institute), and SPSS V.25.0 (SPSS, Chicago, Illinois, USA).

Results

Demographic characteristics of the participants

For further screening, 716 participants refused or were unable to take the BMD measurement. Another 1,517 premenopausal women were excluded from the final analyses because *Z*-scores, but not

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TABLE 1 General characteristics of the participants.

	Total ($n = 19,848$)	Male ($n = 5,643$)	Female ($n = 14,205$)	*P*-value
Age (year), mean (SD)	63.79 (7.11)	65.40 (6.97)	63.16 (7.06)	<0.001
Age, n (%)				
40–49 years	276 (1.39)	67 (1.19)	209 (1.47)	<0.001
50–59 years	5,446 (27.44)	1,031 (18.27)	4,415 (31.08)	
60–69 years	9,740 (49.07)	2,925 (51.83)	6,815 (47.98)	
70–79 years	4,244 (21.38)	1,561 (27.66)	2,683 (18.89)	
80- years	142 (0.72)	59 (1.05)	83 (0.58)	
BMI[a] (kg/m^2), median (P25, P75; n = 19,459)	24.02 (21.99, 26.17)	24.38 (22.49, 26.40)	23.83 (21.79, 26.06)	<0.001
BMI[a], n (%) ($n = 19,459$)				
<18.5 kg/m^2	598 (3.07)	128 (2.32)	470 (3.37)	<0.001
18.5–24.9 kg/m^2	11,570 (59.46)	3,064 (55.59)	8,506 (60.99)	
≥25.0 kg/m^2	7,291 (37.47)	2,320 (42.09)	4,971 (35.64)	
Education[b] (n = 18,877)				
No formal education	1,035 (5.48)	98 (1.85)	937 (6.90)	<0.001
Primary school	2,915 (15.44)	760 (14.34)	2,155 (15.87)	
Middle school	5,706 (30.23)	1,676 (31.62)	4,030 (29.68)	
High school	6,104 (32.34)	1,459 (27.52)	4,645 (34.21)	
College	1,927 (10.21)	707 (13.34)	1,220 (8.99)	
University or higher	1,190 (6.30)	601 (11.34)	589 (4.34)	
Smoking status[c] (n = 18,696)				
Never	14,444 (77.26)	2,589 (47.97)	11,855 (89.15)	<0.001
Former	1,007 (5.39)	910 (16.84)	97 (0.73)	
Current regular	1,996 (10.68)	1,732 (32.07)	265 (1.99)	
Passive smoking	1,249 (6.68)	168 (3.11)	1,081 (8.13)	
Region, n (%)				
Shanghai	5,810 (29.27)	1,740 (30.84)	4,069 (28.64)	<0.001
Beijing	2,784 (14.03)	762 (13.51)	2,022 (14.23)	
Guangdong	3,376 (17.01)	813 (14.41)	2,563 (18.04)	
Jilin	1,626 (8.19)	443 (7.85)	1,183 (8.33)	
Gansu	2,724 (13.72)	707 (12.53)	2,017 (14.20)	
Yunnan	1,780 (8.97)	692 (12.27)	1,088 (7.66)	
Jiangxi	1,748 (8.81)	485 (8.60)	1,263 (8.89)	
History of fracture[d], *n* (%)	3,657 (18.44)	944 (16.75)	2,713 (19.11)	<0.001
Hyperlipidemia, *n* (%)	3,810 (19.20)	966 (17.12)	2,844 (20.02)	<0.001
Hypertension, *n* (%)	6,729 (33.90)	2,036 (36.07)	4,693 (33.04)	<0.001
Diabetes, *n* (%)	2,352 (11.85)	791 (14.00)	1,561 (10.99)	<0.001
Lumbar BMD[e], mean (SD)	0.86 (0.17)	0.96 (0.17)	0.83 (0.15)	<0.001
Hip BMD[f], mean (SD)	0.81 (0.14)	0.89 (0.14)	0.77 (0.13)	<0.001
Bone mass category[g], n (%)				
Osteoporosis	6,599 (33.39)	1,090 (19.39)	5,509 (38.96)	<0.001
Osteopenia	7,535 (38.13)	2,042 (36.31)	5,493 (38.85)	

(Continued)

TABLE 1 (Continued)

	Total ($n = 19,848$)	Male ($n = 5,643$)	Female ($n = 14,205$)	*P*-value
Bone mass category[h], n (%)				
Osteoporosis	2,032 (11.85)	213 (4.36)	1,819 (14.85)	<0.001
Osteopenia	8,126 (47.41)	2,083 (42.60)	6,043 (49.33)	
Bone mass category[i], n (%)				
Osteoporosis	7,051 (35.52)	1,151 (20.40)	5,900 (41.53)	<0.001
Osteopenia	8,330 (41.97)	2,464 (43.65)	5,866 (41.30)	

[a]There were 389 missing values for body mass index (BMI).
[b]There were 971 missing values for education level.
[c]There were 1,152 missing values for smoking status.
[d]There were 13 missing values for history of fracture.
[e]There were 197 missing values for lumbar BMD.
[f]There were 197 missing values for hip BMD.
[g]Diagnosed by lumbar spine BMD.
[h]Diagnosed by hip BMD.
[i]Diagnosed by BMD of lumbar spine or hip.

T-scores of BMD are used for the diagnosis of osteoporosis in premenopausal women. Finally, 19,848 participants (90%) were enrolled for the final analysis. The general characteristics of the study population were presented in Table 1. The average age of all the participants was 63.79 ± 7.11, among which 28.43% were men. BMD values of men in both lumbar spine and hip were higher than those of women ($P < 0.001$). The proportion of osteoporosis diagnosed by hip BMD was significantly lower than that by lumbar spine BMD ($P < 0.001$).

Serum levels of bone metabolic markers and calcium and phosphorus metabolism indicators

The serum concentrations of bone metabolic markers, and calcium and phosphorus metabolism indicators listed in the Supplementary Table S1 were influenced by age, BMI, gender, education level, regions and bone mass status. Among women, the serum concentrations of P, Ca, PINP, β-CTX, OST, ALP, and PTH were significantly higher than those of men, while 25(OH)D of men was higher than that of women ($P < 0.001$). The expression levels of these markers and indicators also showed significant differences among people of different ages ($P < 0.001$). Serum levels of PINP and OST as well as Ca, P, and 25(OH)D showed peak values in the participants of 50–59 years old, followed by gradual decreasing trends with aging, while bone resorption marker β-CTX showed a continuous increasing trend along with intensified aging process. Serum levels of PTH and another bone formation marker ALP also displayed upward trends until the years of 70–79. As BMI increased, the serum concentrations of P, PINP, OST, β-CTX, and 25(OH)D decreased ($P < 0.001$), while ALP and PTH increased ($P < 0.001$) suggesting relative higher bone turnover of people with lower BMI. Along with increased education level, serum concentrations of PINP, OST, and ALP showed overall downward trends indicating decreased bone formation in people with higher education level. The serum levels of these markers and indicators were also different among people of different regions ($P < 0.001$). Shanghai had the lowest concentration of serum PINP and the highest concentration of serum β-CTX, and Jiangxi had the highest concentration of serum PINP and OST. Guangdong showed the lowest concentration of serum ALP and PTH and the highest 25(OH)D, and Gansu had the highest ALP and lowest 25(OH)D. The serum concentrations of all the bone metabolic markers as well as calcium and phosphorus in osteoporosis group were higher than those in both osteopenia group and normal group ($P < 0.01$).

Estimated prevalence of osteoporosis in the middle aged and elderly people in China

Estimated prevalence of osteoporosis was listed in Table 2. The age-standardized prevalence of osteoporosis was estimated to be 33.49% (95% CI, 32.80–34.18%) in the middle-aged and elderly Chinese permanent residents, 20.73% (95% CI, 19.58–21.87%) in men, and 38.05% (95% CI, 37.22–38.89%) in women. The prevalence of osteoporosis was increased with aging in women. For men, the prevalence of osteoporosis was 23.47, 19.10, 20.99, and 23.73% respectively in their 50, 60, 70, and 80 s and older. For women, the prevalence of osteoporosis was 13.39, 25.99, 38.67, 48.78, and 57.75% in their 40, 50, 60, 70, and 80 s and older (Supplementary Table S2). In addition, the prevalence of osteoporosis showed a downward trend with the increase of education level in women with education level lower than college, and the prevalence showed a similar trend with BMI in both men and women (Supplementary Table S2). There was a high prevalence of osteoporosis in current regular smoking participants in men (23.01%; 95% CI, 20.90–25.12%, Supplementary Table S2). The estimated prevalence of osteoporosis among total population also varied regionally, with the lowest prevalence in the east (Shanghai, 26.21%; 95% CI, 25.00–27.39%) and the highest in the south-east (Jiangxi, 54.58%; 95% CI, 52.06–57.10%). The prevalence of osteoporosis of the south-west (Yunnan), the west (Gansu), the north-east (Jilin), the south (Guangdong), and the north (Beijing) was 45.15, 33.49, 32.65, 31.55, and 29.11%, respectively. The

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TABLE 2 Estimated prevalence of osteoporosis and osteopenia in each group.

	Normal ($n = 4,467$)	Osteopenia ($n = 8,330$)	Osteoporosis ($n = 7,051$)
Overall	23.27 (22.63, 23.90)	43.24 (42.50, 43.98)	33.49 (32.80, 34.18)
Age			
40–49 years ($n = 276$)	35.43 (29.75, 41.11)	45.29 (39.37, 51.20)	19.29 (14.58, 23.98)
50–59 years ($n = 5,446$)	24.79 (23.64, 25.93)	46.43 (45.10, 47.75)	28.79 (27.58, 29.99)
60–69 years ($n = 9,739$)	21.91 (21.07, 22.75)	41.97 (40.97, 42.97)	36.12 (35.15, 37.09)
70–79 years ($n = 4,244$)	20.53 (19.30, 21.76)	36.88 (35.42, 38.35)	42.59 (41.08, 44.09)
80- years ($n = 142$)	18.07 (11.84, 24.30)	34.86 (26.89, 42.82)	47.07 (38.69, 55.45)
Gender			
Men ($n = 5,642$)	34.57 (33.25, 35.89)	44.70 (43.30, 46.11)	20.73 (19.58, 21.87)
Women ($n = 14,205$)	19.23 (18.51, 19.94)	42.72 (41.85, 43.59)	38.05 (37.22, 38.89)
BMI			
<18.5 kg/m^2 ($n = 598$)	6.10 (3.90, 8.30)	26.47 (22.51, 30.43)	67.43 (63.24, 71.62)
18.5–24.9 kg/m^2 ($n = 11,570$)	17.57 (16.81, 18.32)	43.79 (42.82, 44.77)	38.64 (37.69, 39.59)
≥ 25.0 kg/m^2 ($n = 7,291$)	33.69 (32.54, 34.83)	44.11 (42.92, 45.31)	22.20 (21.23, 23.18)
Education			
No formal education	13.05 (10.78, 15.32)	34.60 (31.45, 37.75)	52.36 (49.07, 55.64)
Primary school	17.63 (16.13, 19.14)	40.07 (38.16, 41.98)	42.30 (40.38, 44.22)
Middle school	23.51 (22.33, 24.68)	44.99 (43.52, 46.46)	31.46 (30.21, 32.72)
High school	24.70 (23.55, 25.84)	44.76 (43.45, 46.07)	30.54 (29.34, 31.75)
College	27.61 (25.46, 29.76)	44.11 (41.74, 46,48)	28.28 (26.18, 30.39)
University or higher	27.81 (25.05, 30.57)	42.68 (39.60, 45.76)	29.51 (26.69, 32.34)
Region			
Shanghai	31.71 (30.42, 33.00)	42.08 (40.72, 43.45)	26.21 (25.00, 27.39)
Beijing	25.03 (23.28, 26.79)	45.86 (43.88, 47.83)	29.11 (27.35, 30.87)
Guangdong	23.10 (21.59, 24.60)	45.35 (43.59, 47.10)	31.55 (29.94, 33.17)
Jilin	23.18 (20.93, 25.43)	43.33 (40.76, 45.90)	33.49 (31.11, 35.87)
Gansu	20.33 (18.71, 21.95)	47.02 (45.03, 49.00)	32.65 (30.81, 34.49)
Yunnan	14.04 (12.25, 15.83)	40.82 (38.33, 43.31)	45.15 (42.66, 47,63)
Jiangxi	10.98 (9.34, 12.63)	34.44 (32.01, 36.86)	54.58 (52.06, 57.10)
Smoking status[a] ($n = 18,695$)			
Never	24.04 (23.18, 24.9)	44.59 (43.61, 45.58)	31.37 (30.46, 32.27)
Former	40.25 (36.63, 43.86)	43.49 (39.8, 47.17)	16.26 (13.57, 18.96)
Current regular	29.88 (27.54, 32.23)	47.30 (44.74, 49.87)	22.82 (20.68, 24.96)
Passive smoking	21.81 (19.18, 24.43)	46.57 (43.41, 49.72)	31.63 (28.73, 34.53)
History of fracture ($n = 3,657$)	17.27 (15.94,18.60)	42.91 (41.19, 44.64)	39.82 (38.13, 41.50)
Hyperlipidemia			
Yes	26.76 (25.24,28.28)	42.57 (40.90, 44.25)	30.66 (29.13,32.20)
No	22.48 (21.79,23.18)	43.39 (42.57,44.21)	34.13 (33.35,34.90)
Hypertension			
Yes	27.37 (26.22,28.53)	43.19 (41.92, 44.47)	29.43 (28.28,30.57)
No	21.48 (20.72,22.24)	43.26 (42.36,44.16)	35.26 (34.40,36.11)

(Continued)

TABLE 2 (Continued)

	Normal ($n = 4,467$)	Osteopenia ($n = 8,330$)	Osteoporosis ($n = 7,051$)
Diabetes			
Yes	31.05 (29.01,33.08)	41.30 (39.16, 43.44)	27.65 (25.74,29.57)
No	22.36 (21.70,23.03)	43.47 (42.68,44.25)	34.17 (33.43,34.91)

[a]There were 1,152 missing values for smoking status.

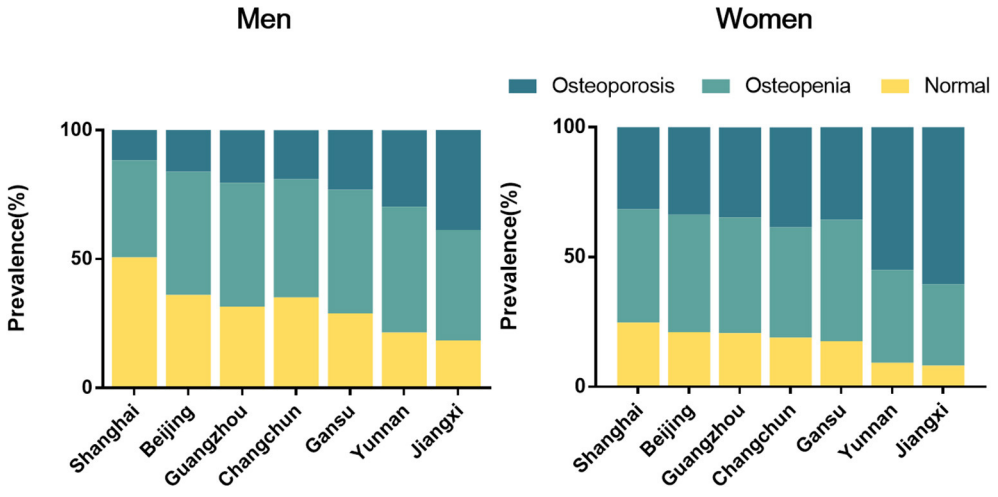

FIGURE 2
Prevalence of osteopenia and osteoporosis in the middle-aged and elderly people in different regions of China by sexual.

respective prevalence of osteoporosis in man and women also showed the similar results (Figure 2). The prevalence of osteoporosis was also significantly increased among participants with a history of previous fractures. But a lower prevalence of osteoporosis was found in people with chronic diseases such as hypertension, hyperlipidemia, or diabetes compared with those without the chronic diseases (Supplementary Table S2).

Risk factors for osteoporosis and osteopenia in the middle aged and elderly people in China

In the multivariable multinomial logit models, compared with those in Shanghai, the middle-aged and elderly people in other provinces were more susceptible to osteoporosis and osteopenia. In addition, women, aged 60 or above, BMI lower than 18.5 kg/m^2; low education level including middle school, primary school and no formal education as well as current regular smoking, a history of fracture were all significantly associated with a higher risk of osteoporosis and osteopenia in the middle-aged and elderly people. BMI equal to or lower than 25.0 kg/m^2 and a history of diabetes were associated with lower risks of both osteoporosis and osteopenia, and a history of hypertension was associated with a lower risk of osteoporosis (Table 3).

Discussion

There are often no characteristic manifestations or only back pain in the early stage of the disease, and patients and even clinicians are prone to ignore the disease. Most patients are diagnosed only after passive physical examination or brittle fracture. Therefore, understanding the risk factors of osteoporosis is beneficial to the early diagnosis and prevention of osteoporosis.

China is the largest developing country in the world, constituting one-fifth of the global population and even higher percentage of the elderly population. The epidemiology of osteoporosis in China had its own unique features compared with that in Western countries. Nationwide studies on epidemiology of osteoporosis, especially in Chinese mainland are scarce. According to the results of 2020 population census of China released by the National Bureau of Statistics, people aged 60 or above accounts for 18.7% of the total population, which was only 13.26% in 2010, and the number of people aged 60 or above has been increased over 86 million in the past 10 years (14). Our study confirmed that aged 60 or above was an important risk factor of osteoporosis and osteopenia. According to the estimated prevalence of osteoporosis in our study, there would be more than 100 million aged people suffering osteoporosis, and the number will keep rising. However, a large number of patients with osteoporosis were still far from being fully evaluated and managed.

The respondents came from two municipalities, including Beijing and Shanghai, and five provinces across China including Guangzhou

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TABLE 3 Risk factors for osteoporosis and osteopenia by univariate and multivariate logistic regression analyses.

Risk factors	Osteopenia OR (95% CI)	*P*-value[a]	Osteopenia OR (95% CI)	*P*-value[b]	Osteoporosis OR (95% CI)	*P*-value[a]	Osteoporosis OR (95% CI)	*P*-value[b]
Region								
Shanghai	1.00		1.000		1.000		1.000	
Beijing	1.398 (1.248, 1.567)	<0.001	1.607 (1.413, 1.827)	<0.001	1.476 (1.306, 1.669)	<0.001	2.088 (1.790, 2.437)	<0.001
Guangdong	1.504 (1.35, 1.677)	<0.001	1.565 (1.382, 1.772)	<0.001	1.659 (1.477, 1.863)	<0.001	1.959 (1.690, 2.270)	<0.001
Jilin	1.457 (1.261, 1.683)	<0.001	1.552 (1.326, 1.817)	<0.001	1.904 (1.639, 2.211)	<0.001	2.136 (1.779, 2.565)	<0.001
Gansu	1.727 (1.532, 1.947)	<0.001	1.917 (1.676, 2.193)	<0.001	1.950 (1.718, 2.214)	<0.001	2.702 (2.306, 3.166)	<0.001
Yunnan	2.121 (1.806, 2.49)	<0.001	2.973 (2.486, 3.557)	<0.001	3.916 (3.339, 4.594)	<0.001	6.981 (5.712, 8.533)	<0.001
Jiangxi	2.562 (2.049, 3.202)	<0.001	2.646 (2.048, 3.418)	<0.001	6.682 (5.390, 8.285)	<0.001	6.823 (5.193, 8.964)	<0.001
Gender								
Men	1.000		1.000		1.000		1.000	
Women	1.989 (1.841, 2.148)	<0.001	2.360 (2.123, 2.622)	<0.001	4.375 (4.000, 4.784)	<0.001	6.060 (5.289, 6.943)	<0.001
Age, year								
<60	1.000		1.000		1.000		1.000	
≥60	1.034 (0.955, 1.120)	0.403	1.452 (1.318, 1.599)	<0.001	1.610 (1.478, 1.755)	<0.001	2.673 (2.382, 2.998)	<0.001
BMI, kg/m^2								
<18.5	1.685 (1.141, 2.488)	0.009	1.867 (1.221, 2.857)	0.004	5.224 (3.629, 7.520)	<0.001	5.927 (3.909, 8.988)	<0.001
18.5–24.9	1.000		1.000		1.000		1.000	
≥25.0	0.544 (0.504, 0.587)	<0.001	0.506 (0.465, 0.550)	<0.001	0.306 (0.282, 0.333)	<0.0001	0.272 (0.246, 0.301)	<0.001
Education								
No formal education	1.867 (1.461, 2.386)	<0.001	1.728 (1.314, 2.273)	<0.001	4.151 (3.256, 5.293)	<0.001	2.765 (2.039, 3.750)	<0.001
Primary school	1.562 (1.313, 1.857)	<0.001	1.676 (1.374, 2.044)	<0.001	2.362 (1.971, 2.831)	<0.001	2.263 (1.784, 2.871)	<0.001
Middle school	1.323 (1.135, 1.542)	<0.001	1.395 (1.169, 1.664)	<0.001	1.388 (1.177, 1.636)	<0.001	1.443(1.161, 1.792)	0.001
High school	1.230 (1.056, 1.432)	0.008	1.241 (1.042, 1.478)	0.016	1.252 (1.063, 1.474)	0.007	1.217 (0.981, 1.509)	0.074
College	1.087 (0.911, 1.297)	0.353	1.089 (0.895, 1.325)	0.395	1.072 (0.886, 1.296)	0.476	1.015 (0.797, 1.293)	0.901
University or higher	1.000		1.000		1.000		1.000	
Smoking status								
Never	1.000		1.000		1.000		1.000	
Former	0.567 (0.488, 0.658)	<0.001	1.015 (0.855, 1.204)	0.866	0.296 (0.247, 0.355)	<0.0001	0.937 (0.774, 1.134)	0.503
Current regular	0.815 (0.726, 0.915)	0.001	1.339 (1.165, 1.540)	<0.001	0.502 (0.441, 0.572)	<0.0001	1.604 (1.341, 1.920)	<0.001
Passive smoking	1.014 (0.865, 1.188)	0.864	0.965 (0.816, 1.142)	0.678	0.939 (0.798, 1.106)	0.451	0.989 (0.786, 1.245)	0.927
History of fracture								
No	1.000		1.000		1.000		1.000	
Yes	1.402 (1.264, 1.555)	<0.001	1.386 (1.241, 1.550)	<0.001	1.843 (1.661, 2.045)	<0.001	1.762 (1.555, 1.996)	<0.001
Hyperlipidemia								
No	1.000		1.000		1.000		1.000	
Yes	0.851 (0.777, 0.932)	0.001	0.917 (0.826, 1.017)	0.102	0.793 (0.721, 0.872)	<0.001	0.939 (0.830, 1.061)	0.312
Hypertension								
No	1.000		1.000		1.000		1.000	
Yes	0.786 (0.728, 0.848)	<0.001	0.955 (0.873, 1.044)	0.312	0.673 (0.621, 0.729)	<0.001	0.812 (0.731, 0.903)	<0.001
Diabetes								
No	1.000		1.000		1.000		1.000	
Yes	0.707 (0.635, 0.786)	<0.001	0.746 (0.662, 0.840)	<0.001	0.603 (0.538, 0.676)	<0.001	0.705 (0.611, 0.814)	<0.001

[a]Univariate linear regression model without adjustment.

[b]Multivariate logistic regression analyses adjustment for province, sex, age, BMI, education, smoking status, history of fracture, hyperlipidemia, hypertension, and diabetes as independent variables.

and Shenzhen in Guangdong province, Ganzhou in Jiangxi Province, Lanzhou in Gansu Province, Changchun in Jilin Province and Kunming in Yunnan Province. The distribution represents the north, east, south, west, north-east, south-east, and south-west regions of China with representative features in climate, environment and lifestyles. In recent overview of osteoporosis, a higher prevalence among residents in northern China was reported (15). And Zeng's study indicated that BMD values at the spine and hip were significantly lower in the participants from Southwest China than in those from other geographic regions (3). In our study, we included more regions of China, and found that the prevalence of osteoporosis of the inland regions including the southeast, the southwest, the northwest and the northeast were relatively higher than that of the eastern coastal regions. Multiple region-related factors, such as genetic predisposition, latitude and longitude (3), diet (16, 17), sunlight (18), physical activities (19), lifestyles (20), socioeconomic status (21), and even environment pollution (22, 23), could contribute to the differences in the prevalence of different regions of China.

Educational level is one of the most important socioeconomic indicators and closely related to the income level. People with higher education levels usually have stronger health awareness and more health knowledge, living in a healthier lifestyle with better medical resources. It contributes to the decreased prevalence of osteoporosis along with increased education level in the middle-aged and elderly people. Studies also had confirmed that knowledge awareness of osteoporosis was significantly correlated with BMD in postmenopausal women (5, 24).

Chronic metabolic diseases with high incidences in China including hypertension, hyperlipidemia and diabetes affect the development of osteoporosis physically (10–12). However, our study revealed a protective factor of diabetes on osteopenia and osteoporosis. Some large clinical studies (25, 26) also found that patients with diabetes had preserved or even increased BMD compared with control individuals without diabetes. The authors postulated that additional factors, including altered adipokine levels such as increased leptin and reduced adiponectin, and hyperinsulinemia might potentially have mediated the effects of obesity on BMD in diabetes. In addition, insulin resistance might also contribute to the reduced bone turnover and higher BMD in diabetes (27). These findings have been subsequently confirmed and extended by the meta-analysis to date on the association between BMD and diabetes. Despite higher levels of BMD than individuals who do not have diabetes, patients with diabetes tend to sustain fragility fractures with impaired bone material properties seeming to most consistently contribute to skeletal frailty in patients with diabetes. Alterations in bone material properties are at least partially related to glycation end products accumulation (28), which may be caused by the usage of insulin or other hypoglycemic drugs (29).

In addition to the influence of aging, region, education level, and history of chronic diseases, we also confirmed a higher prevalence in women and people with low BMI or a history of fracture which was consistent with previous findings (3, 13). The prevalence of osteoporosis in men over 60 years old is about one-third of that of women. The gradually decreased androgen levels associated with aging, decreased exposure to physical exercises and social activities may contribute to the occurrence of osteoporosis in the elderly men. In addition to decreased physical exercises and social activities, menopause occurs with the rapid decrease in estrogen level, which causes over activated osteoclast formation and imbalanced bone metabolism in the elderly women, and finally leads to dramatic bone loss (30). Also, a previous study suggested that nutrient deficiencies, such as vitamins, are common in older women (31). Low intake of daily protein and vitamins also contributes to bone dystrophy and accelerated bone loss.

According to the Chinese society of Osteoporosis and Mineral Research (CSOBMR) criteria, osteoporosis of postmenopausal women and man over 50 years old was defined as a *T*-score of BMD ≤ -2.5 SD (32). It was reported that the BMD values of lumbar spine were lower than that of hip in age and gender matched groups (3), and spine is the site of rapid bone loss compared with hip. The present study also confirmed that the prevalence of osteoporosis would be greatly reduced if diagnosing by BMD of each single region only. Also, the prevalence of osteoporosis was dramatically reduced according to BMD of hip. To screen out more patients with osteoporosis, it is more appropriate to make a diagnosis when *T*-score of BMD of any part ≤ -2.5 SD.

In order to achieve the early diagnosis and prevention of osteoporosis, it is important to identify and pay close attention to the high-risk population of osteoporosis. In this study, the regional differences in osteoporosis prevalence in China were revealed. It was also proved that women, aged 60 or above, BMI lower than 18.5 kg/m^2, low education level including middle school, primary school and no formal education as well as current regular smoking, a history of fracture are associated with a higher risk of osteoporosis and osteopenia in the middle-aged and elderly people of China. These findings are helpful to formulate the screening program and local policies for prevention and treatment of osteoporosis.

The present study had some strength. Firstly, compared to previous studies on osteoporosis prevalence in China (3, 33), we include some major demographic and socioeconomic factors, such as age, gender, education levels, history of chronic diseases, which were confirmed to be associated with the occurrence of bone loss and osteoporosis. So far, it was the first study to observe the relationship between these factors and the risk of osteoporosis at the same time in China. It provided basis of which more prevention and treatment resources should be invested into particular population exposed to specific factors. Secondly, the DXA instruments used in all centers were of the same model, and the BMD values of both lumbar spine and hip were collected. Therefore, the BMD reference database established in the present study was comparable, which was largely improved compared with the previous studies of BMD measurements in the Chinese population (3, 34, 35).

Nevertheless, there were also several limitations. First of all, the participants in our study were volunteers from multiple regions, but not randomly selected, which might cause selection bias. Secondly, the participants who were already on anti-osteoporosis treatment were not excluded, and it might cause a lower prevalence than it actually was. Third, considering the time costs and practical operability, some other factors, such as dietary intake, medical history and physical activities which might also affect BMD values at certain degree were not collected in the study.

Conclusion

This multicenter survey revealed dramatic regional differences in osteoporosis prevalence in China, and woman, aged 60 years or older, low BMI, low education level, and current regular smoking might contribute to the occurrence of osteoporosis. More prevention and treatment resources should be invested into particular population exposed to these risk factors.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board at Longhua Hospital affiliated to the Shanghai University of Traditional Chinese Medicine. The patients/participants provided their written informed consent to participate in this study.

Author contributions

All authors contributed to the study's conception and design, as well as the acquisition, analysis, interpretation of data, drafting and revising of the article, and agreed to be accountable for all aspects of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1084005/full#supplementary-material

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Effects of resistance and balance exercises for athletic ability and quality of life in people with osteoporotic vertebral fracture: Systematic review and meta-analysis of randomized control trials

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Purpose: This study aimed to use meta-analysis to determine the impact of resistance and balance training on athletic ability and quality of life for patients with osteoporotic vertebral fracture (OVF).

Methods: This study followed the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) criteria for systematic reviews and meta-analyzes. The PubMed, Web of science, Cochrane, Embase, and CNKI databases were searched for randomized controlled trials (RCTs) up to September 2022. The search strategy was related to the intervention measures, population, and results, and was structured around the search terms: "Exercise," "Osteoporotic vertebral fracture," and "activities of function." Two reviewers strictly implemented the inclusion and exclusion criteria. Subgroup analyzes of age and training duration were performed for the main outcomes.

Results: We included 12 RCTs (n=1,289) of resistance and balance training in patients with OVF. Compared with controls, the intervention group showed improvements on the Quality of Life Questionnaire issued by the European Foundation for Osteoporosis, visual analog pain scale, Timed Up and Go, falls efficacy scale international (FES-I), kyphosis, and functional reach. On subgroup analysis, the effect was more significant when training continued >10weeks.

Conclusion: Resistance and balance exercise training improved function and balance, and reduced fall risk in patients with OVF. We recommend resistance and balance training for at least 10weeks. Future multicenter, large sample trials are needed for more reliable conclusions.

KEYWORDS

resistance and balance exercise, osteoporotic vertebral fracture, functional status and balance, randomized control trials, systematic review and meta-analysis

Introduction

Osteoporosis is a metabolic bone disease characterized by decreased bone mass and deterioration of bone tissue microstructure that is common around the world (1). Osteoporotic vertebral fracture (OVF) is one of the most common consequences of osteoporosis (2). A cross-sectional study of the Chinese mainland found that the prevalence of osteoporosis in men and women over 40 years old was 5.0% and 26.0%, respectively, and that OVF occurred in 10.5% of men and 9.7% of women. Although this incidence of osteoporosis and fracture in China is very high, few patients have received osteoporosis treatment; thus, it has been considered a "silent 21st century epidemic" (3, 4). However, its treatment cost remains huge. In the United States, the high medical resource utilization rate and medical costs of OVF have far exceeded the costs of stroke, myocardial infarction, and breast cancer (5). OVF can result in loss of height, acute and chronic pain, impaired ambulation/balance, decreased quality of life, and shortened life span (6). More importantly, OVF can also lead to increased future re-fracture risk (7, 8). Falls have been considered the primary risk of fracture in patients with OVF (9). Therefore, reducing falls and improving body balance in patients with OVF are considered important measures to reduce re-fracture.

Exercise can delay the negative effects of chronic aging diseases on the body (10). For osteoporosis, exercise is a safe and low-cost non-medication intervention (11). Exercise can reduce the estimated loss by maintaining the cortical and trabecular bone density. It can also improve patient function, including exercise ability and balance, and back muscle strength (12, 13). Therefore, it is strongly recommended that patients with osteoporosis take part in exercise, especially balance and resistance strength training (14). One study found that resistance and balance exercises significantly enhanced lumbar muscle strength, reduce bone loss, and decreased lumbar fracture incidence in postmenopausal women (15). Is exercise beneficial for patients with OVF? A literature search revealed one previously published systematic review on the impact of exercise on patients with OVF, which was unable to determine clear benefits of exercise in people with spinal fractures (16). However, the investigation was assessing simple exercise rather than a specific type of training. In recent years, resistance and balance training have been gradually applied as a composite exercise program to intervene for OVF. Nevertheless, a specific relationship of resistance and balance exercise with OVF has not been previously quantified. It is essential to clarify the specific therapeutic effects (e.g., enhancing motor and balance function, reducing back pain and fear of falling) of resistance and balance training for patients with OVF, because it could impact their rapid rehabilitation significantly. Therefore, we conducted this systematic review and meta-analysis evaluating the use of resistance and balance exercise training for OVF.

Information sources and search strategy

The referenced data was searched in the following electronic databases: PubMed, Web of Science, Cochrane, Embase and CNKI. We systematically searched the above databases for articles published up until September 17, 2022, without language restrictions. The search strategy was related to intervention measures, population, and results, and was structured around the search terms: "Exercise," "Osteoporotic vertebral fracture" and "Functional activities." Keywords and their synonyms were used to improve search sensitivity: ("Exercises" OR "Activities, Physical" OR "Activity, Physical" OR "Physical Activities" OR "Exercise, Physical" OR "Exercises, Physical" OR "Physical Exercise" OR "Physical Exercises" OR "Exercise Training" OR "Exercise Training" OR "Training, Exercise" OR "Training, Exercise") AND ("Osteoporotic fracture" OR "Fractures, Osteoporotic" OR "Fracture, Osteoporotic") AND ("Fracture, Spinal" OR "Fractures, Spinal" OR "Spinal Fracture") AND ("Function" OR "Activities of daily living" OR "Functioning"). In PubMed, search results were limited to "randomized controlled trials." Search strategy in Supplementary Document. The first author (LXF) screened studies by title and abstracts according to the inclusion and exclusion criteria. In addition, a manual search in the references and abstracts of all included articles and previous relevant systematic reviews and meta-analyzes was carried out. The standards of the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guided this systematic review and meta-analysis (17).

Inclusion criteria

The participants, intervention, comparison, outcome, time, and study design (PICOTS) criteria were considered to determine the study inclusion criteria: (1) The participants had been diagnosed with osteoporosis and suffered at least one vertebral fracture, verified by DXA-based vertebral fracture assessment or X-ray by medical doctors in a clinical setting, (2) The intervention was standardized progressive exercise therapy, especially resistance and balance training, (3) The control group maintained their previous level of daily and physical activities, (4) The outcomes were patients' balance, mobility, and health-related quality of life, using measures including the "Quality of Life Questionnaire issued by the European Foundation for Osteoporosis" (QUALEFFO-41), Timed Up and Go (TUG), walking speed, VAS (visual analog pain scale), kyphosis, time-loaded standing, etc., and (5). The study design was RCTs published in authoritative journals.

Exclusion criteria

We excluded studies with the following characteristics: (1) Full text and/or data inaccessible, (2) Participants with other bone metabolic diseases (diabetes, thyroid dysfunction), and (3) Patients with cancer for chemo and/or radiotherapy.

Data extraction

Two researchers (CWH, CQ) independently extracted data after reading the full text, and the third investigator (LFF) solved any disagreement. The collected information included the first author's name, publication year, participant characteristics (mean age and gender), sample size, characteristics of exercise intervention (training frequency and intervention duration), risk assessment, and outcome characteristics.

Outcome measures

In this systematic review and meta-analysis, the primary outcomes were scores of QUALEFFO-41, VAS, and functional reach test (FR) assessments. Secondary measures were scores of the TUG and Falls Efficacy Scale International (FES-I) and measurements of kyphosis.

Study quality assessment

Two researchers (CC, LP) independently used Cochrane's collaborative tools (risk of bias) to assess the methodological quality of every RCT. Disagreements were resolved through discussions with the third assessor (LFY). The risk of bias assessment includes random sequence generation, allocation concealment, participants and personnel blinding, outcome assessment blinding, incomplete outcome data, selective reporting, and other bias. All standards were equally estimated with "low," "unclear," and "high" risk levels.

Data synthesis

All analyzes were carried out using Review Manager 5.3 (Nordic Cochrane Center, Copenhagen, Denmark). The extracted results data is completed using changes in the mean and standard deviation (SD) values. Subtract the mean value before the intervention from the mean value after the intervention and calculate the standard deviation of the change according to the number of subjects in the study group combined with the value of group p or 95% confidence interval (the changes of mean value and standard deviation are not reported). The χ^2-test and I^2-value were used to evaluate the heterogeneity of individual research results. The fixed effect model was used when I^2 was less than 50%, and the random effect model was used when I^2 was more than 50%. In addition, subgroup analysis was used to identify potential causes of heterogeneity.

Subgroup analysis and exploring heterogeneity

In cases of heterogeneity, we expect the following subgroup analyzes (*a priori*): patient's age (less than or over 70 years) and duration of exercise intervention (less than or over 10 weeks). We planned to use a funnel chart to evaluate for publication bias.

Results

Study selection

807 studies were initially identified from the selected databases; the document management software automatically deleted 96 duplicate entries. The remaining 711 studies were screened using the title and abstract, excluding another 661 studies. The remaining 50 studies were evaluated according to the inclusion and exclusion standards listed above. Ultimately, 12 RCTs were selected for our meta-analysis (18–29). See Figure 1 for the inclusion flow diagram. Two RCTs (19, 20) were defined as discrete research because of different follow-up times.

Study characteristics and interventions

In 12 RCTs, the total number of participants was 1,289 (exercise group: $n = 666$; control group: $n = 623$) and sample sizes of the individual studies varied from 9 (25) to 216 (23). Of the 12 RCTs, eight (18–21, 24, 26, 28, 29) included only females ($n = 724$); and four (22, 23, 25, 27) included both sexes (M:F = 107:458). Age was closely associated with OVF (30). The mean age of participants in five RCTs (18–21, 23) was over 70 years, and it was lower than 70 years in seven (22, 24–29). All participants had been diagnosed with osteoporosis and had suffered at least one vertebral fracture, verified by MRI or CT. In 12 RCTs, the control groups were instructed to continue their current lifestyle. The intervention methods of the exercise groups were resistance and balance training. Four RCTs (22, 27–29) only evaluated resistance and balance training of the systematic lumbar and back muscles. Eight RCTs (18–21, 23–26) studied resistance and balance training of major muscle groups of the entire body and all four limbs. Three RCTs (18, 21, 26) involved aerobic training. The intervention duration varied from 4 weeks (28) to 1 year (24, 26). The frequency of prescribed training ranged from one session weekly (25) to two sessions daily (28). The prescribed training frequency was not specified in two studies (22, 27). Details of study characteristics and interventions are shown in Table 1.

Methodology quality

The Cochrane Collaboration tool was used to assess the RCT deviation risk on systematic review and meta-analysis. The results of the methodological quality assessment are shown in Figures 2, 3. All studies were judged as low risk of bias in the random sequence generation and selective reporting. Five studies were judged as high-risk of bias in the blinding of participants and personnel (25–29). All studies were judged at low risk of bias in the blinding of outcome assessments, except for one study (28) judged as high-risk. Four studies were judged as high-risk of bias for incomplete outcome data (18–20, 23). Risk of bias assessments are shown in Figures 2, 3.

Outcome measures

Effect of resistance and balance exercise training on QUALEFFO-41

Seven RCTs ($n = 936$) assessed the effects of resistance and balance training on QUALEFFO-41 results (18–20, 23, 25, 26, 29). The seven RCT studies showed great heterogeneity ($I^2 = 80\%$, $p < 0.0001$, $\text{Chi}^2 = 6.31$, $\text{df} = 6$); thus, the random effects model was used for analysis. Random effects analysis showed that resistance and balance training significantly decreased QUALEFFO-41 scores compared with those of the control group (mean deviation, MD: −3.65, 95% CI, −5.99 to −1.32, $p = 0.002$; Figure 4).

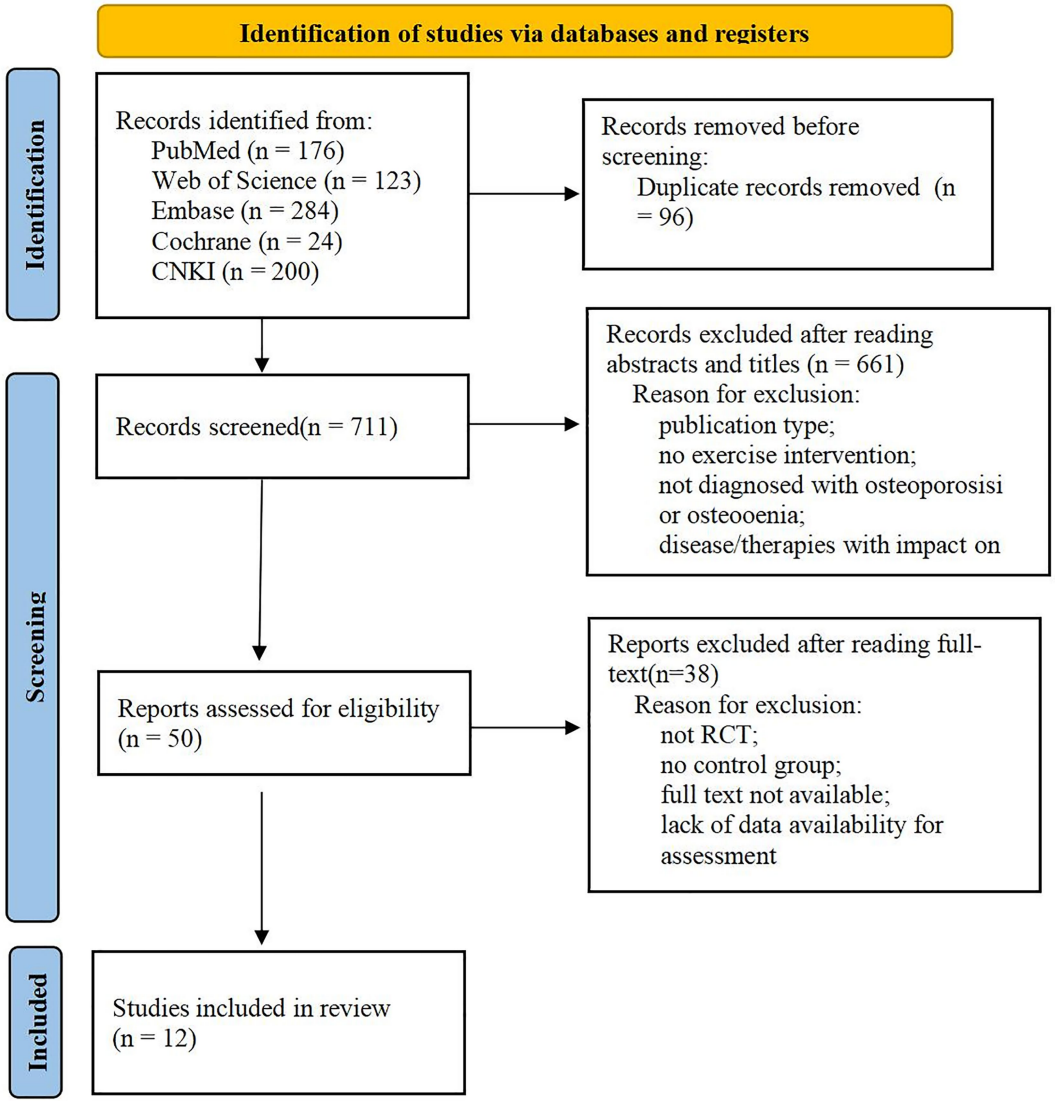

FIGURE 1
PRISMA flow diagram.

Effect of resistance and balance exercise training On functional reach (FRT)

The FRT was used to measure balancing ability. The FRT was used to measure the effects of resistance and balance training in six RCT studies ($n = 917$) (18–21, 23, 28). The heterogeneity among the studies was great ($I^2 = 81\%$, $p < 0.0001$, $Chi^2 = 25.86$, $df = 5$); thus, the random effects model was used for analysis. Random effects analysis showed that the FRT was significantly increased in the group performing resistance and balance training versus the control group (MD: −1.59, 95% CI, −2.61 to −0.58, $p = 0.002$; Figure 5).

Effect of resistance and balance exercise training on VAS

Lumbar back pain was a common complication of OVF. Five RCT studies used a VAS to assess the effect of resistance and balance training ($n = 224$) (22, 25, 27–29). The heterogeneity among the studies was great ($I^2 = 91\%$, $p < 0.00001$, $Chi^2 = 42.60$, $df = 4$); therefore, we used the random effects model for analysis. Random effects analysis showed that the resistance and balance exercise significantly decreased VAS scores in the intervention group compared with controls (MD: −1.59, 95% CI, −2.61 to −0.58, $p = 0.002$; Figure 6).

Effect of resistance and balance exercise training on TUG

The TUG assessed functional mobility. Six RCTs ($n = 357$) assessed the effect of resistance and balance training on "Time Up and Go" ($n = 357$) (18, 24–26, 28, 29). There was great heterogeneity among the studies ($I^2 = 93\%$, $p < 0.0001$, $Chi^2 = 69.74$, $df = 5$); therefore, the random effects model was used for analysis. Random effects analysis showed that TUG significantly decreased in the resistance and balance

TABLE 1 Characteristics of the included RCT studies.

First author, year	Study population	Sample size		Gender		Mean age		Exercise duration	Exercise intervention	Control intervention	Outcome
		Exercisers	Controls	Exercisers	Controls	Exercisers	Controls				
A Bergland, 2010	Women with osteoporosis and at least one vertebral fracture	47	42	F	F	70.8 ± 5.9	72 ± 5.8	2 sessions weekly, 3 months	Aerobic exercise to music (10 min) + Change direction to walk, climb, and avoid obstacles; balance training; chest and trunk exercise and posture promotion (40 min) + Stretching upper and lower limb muscles (10 min)	Maintain current lifestyle	A;B;C
B Stanghelle, 2020	Older women diagnosed with osteoporosis and vertebral fracture	76	73	F	F	74.7 ± 6.1	73.7 ± 5.6	2 sessions weekly, 3 months	Progressive resistance training for all major muscle groups combined with balance training.	Maintain current lifestyle	B;C;H;F
B Stanghelle, 2020	Older women diagnosed with osteoporosis and vertebral fracture	76	73	F	F	74.7 ± 6.1	73.7 ± 5.6	2 sessions weekly, 3 months	Group-based resistance and balance circuit program with instruction; focused on weight-bearing exercises.	Maintain current lifestyle	B;C;H;F
Chen, 2012	Older population with osteoporosis and at least one vertebral fracture	22	20	M:F 3:19	M:F 2:18	70.3 ± 14.1	67.1 ± 15.8	Unspecified	Systematic back muscle exercise with one-point, three-point, and five-point support training.	Maintain current lifestyle	E
C F Olsen, 2014	Older people with osteoporosis and at least one vertebral fracture	47	42	F	F	70.8 ± 5.9	72 ± 5.8	2 sessions weekly, 3 months	Aerobic exercise to music (10 min) + Change direction to walk, climb, and avoid obstacles; balance training; chest and trunk exercise and posture promotion (40 min) + Stretching upper and lower limb muscles (10 min)	Maintain current lifestyle	C; F
Ibolya Mikó, 2016	Older women with osteoporosis and at least one vertebral fracture	50	50	F	F	69.33 ± 4.6	69.1 ± 5.3	3 sessions weekly, 12 months	Combination program of conventional back, lower extremity and torso muscle strengthening and proprioceptive dynamic posture training; with three stages: static, dynamic, and functional phases.	Maintain current lifestyle	A
K L Barker, 2019	Older population with osteoporosis and at least one vertebral fracture	216	195	M:F 31:185	M:F 22:173	72.2 ± 8.4	71.9 ± 9.6	3–5 sessions weekly, 3 months	Pro program, multi-component of balance, strength training, and functional weight-bearing exercise	Single physiotherapy session	B; C; D;

(Continued)

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TABLE 1 (Continued)

First author, year	Study population	Sample size		Gender		Mean age		Exercise duration	Exercise intervention	Control intervention	Outcome
		Exercisers	Controls	Exercisers	Controls	Exercisers	Controls				
Kim L Bennell, 2010	The older people with osteoporosis and at least one spinal fracture	11	9	M:F 4:7	M:F 0:9	66.2 ± 8.0	66.3 ± 11.8	1 session weekly, 10 weeks	Exercise for posture and range of motion, including standing, muscle contraction and extension, and resistance exercise	No additional intervention	A; B; D; E
L. Evstigneeva, 2016	Older women with osteoporosis and at least one vertebral fracture	40	38	F	F	70.7 ± 8.1	67.6 ± 7.0	2 sessions weekly, 12 months	Dynamic training for small and medium-sized muscle groups and limb joints, then dynamic exercise of equal length of major muscle groups and joints, then combined dynamic and breathing exercises	Maintain current level of physical activity	A; B
Wang, 2015	Older population with osteoporosis and at least one vertebral fracture	46	46	M:F 24:22	M:F 21:25	65.76 ± 5.3	66.74 ± 6.5	Unspecified	Progressive functional exercise of low back muscles: three-point and five-point support and flying swallow style training	Maintain current lifestyle	E
Yang, 2007	Older women with osteoporosis and at least one vertebral fracture	15	15	F	F	67.4 ± 5.6	65.6 ± 5.6	2 sessions daily, 4 weeks	Isometric contraction of lower back muscles in lying position; Bending and stretching training and rotation training of the waist in sitting or standing position	Maintain current lifestyle	A; C; E
Yetkin Çergel, 2019	Older women with osteoporosis and at least one vertebral fracture	20	20	F	F	58.90 ± 4.7	59.65 ± 6.5	3 sessions weekly, 6 weeks	Back extensor strengthening, with trunk extension, alternating arm lifts, opposite arm and leg lifts	Maintain daily activities	A; B; D; E

A: Time Up and Go (TUG), B: QUALEFFO-41, C: Functional reach, D: Kyphosis, E: visual analog scale (VAS), F: Falls efficacy scale international (FES-I).

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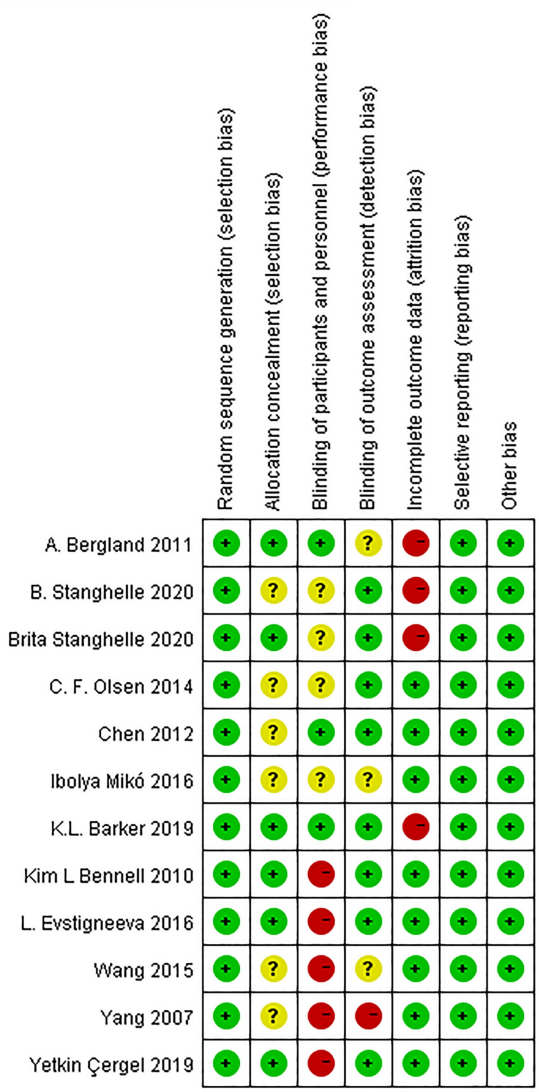

FIGURE 2
Risk of bias assessment summary of RCTs.

exercises group versus controls (MD: −1.98, 95% CI, −3.25 to −0.71, $p = 0.002$; Figure 7).

Effect of resistance and balance exercise training on FES-I

FES-I was used to measure the degree of concern about falls during activities of daily living. Three RCT studies used the FES-I to assess the effect of resistance and balance training ($n = 387$) (19–21). The heterogeneity of the studies was normal ($I^2 = 42\%$); thus, a fixed effects model was used for analysis. Fixed effect analysis showed that FES-I was significantly decreased in the resistance and balance training group compared with the control group (MD: −1.66, 95% CI, −2.89 to −0.43, $p = 0.008$; Figure 8).

Effect of resistance and balance exercise training on kyphosis

Adverse consequences of kyphosis include injurious falls, fractures, functional limitations, mortality, and back pain. Three RCT studies used kyphosis to assess the effect of resistance and balance training ($n = 471$) (23, 25, 29). The heterogeneity among these studies was low ($I^2 = 0\%$); thus, the fixed effect model was used for analysis. Fixed effect analysis showed that kyphosis significantly decreased in the resistance and balance exercise group compared with the control group (MD: −4.79, 95% CI, −8.49 to −1.09, $p = 0.01$; Figure 9).

Subgroup analysis

Age

We conducted subgroup analysis according to age (the mean age of the exercise and control groups combined). The included studies were divided into subgroups of under and over 70 years. Because all VAS studies included had means under 70 years, and those of FES-I were all over 70 years, the outcomes of these two were not included in

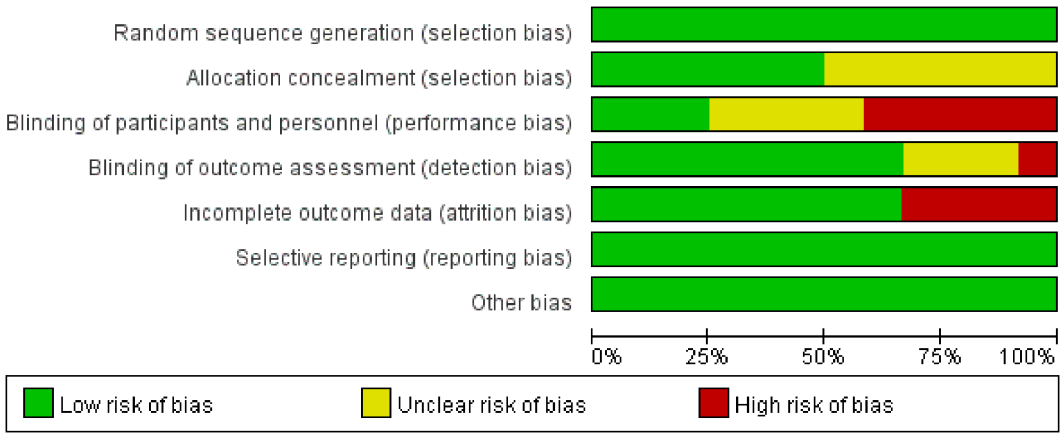

FIGURE 3
Risk of bias items as percentages across all included studies.

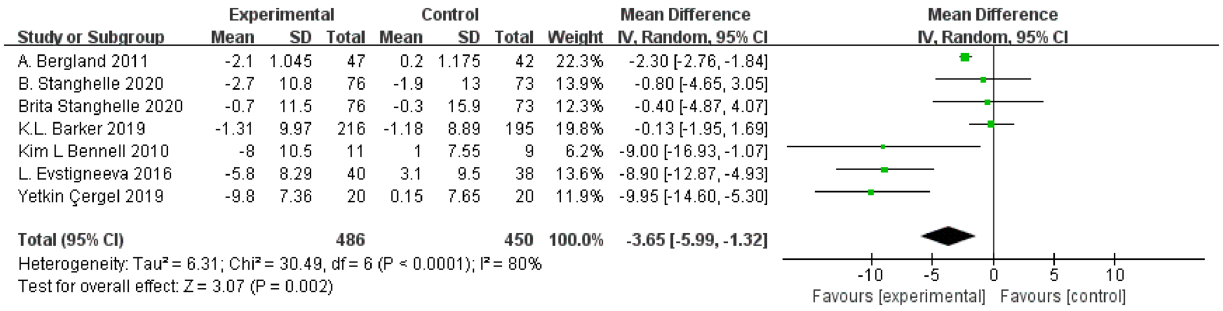

FIGURE 4
Forest plot for QUALEFFO-41 scores.

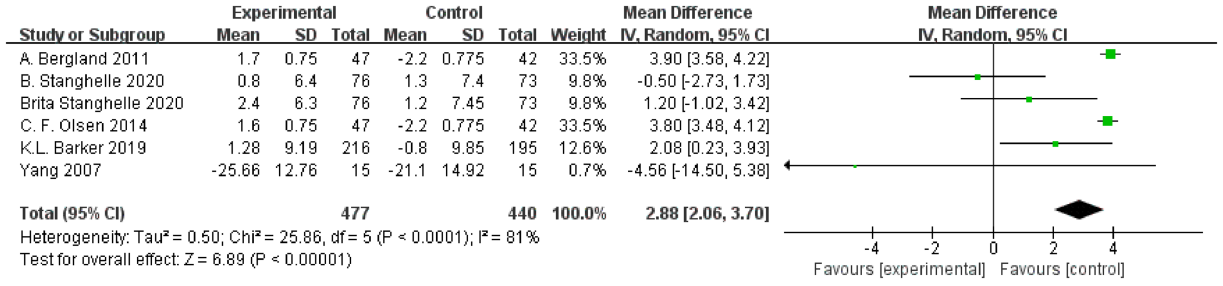

FIGURE 5
Forest plot for functional reach test (FRT).

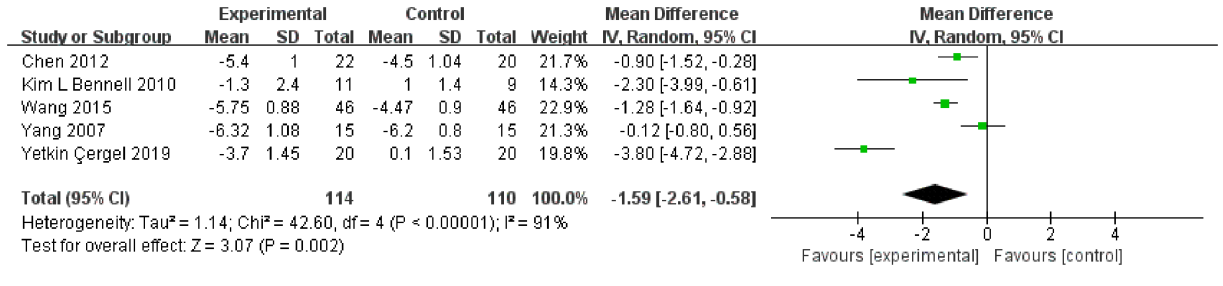

FIGURE 6
Forest plot for the VAS.

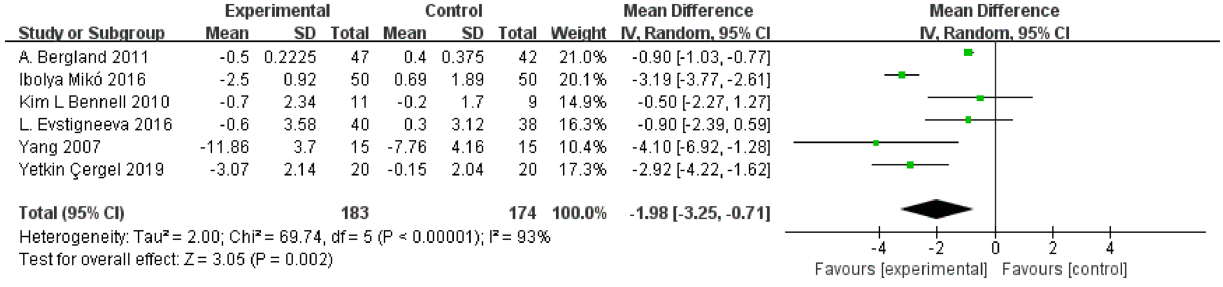

FIGURE 7
Forest plot for Timed Up and Go (TUG).

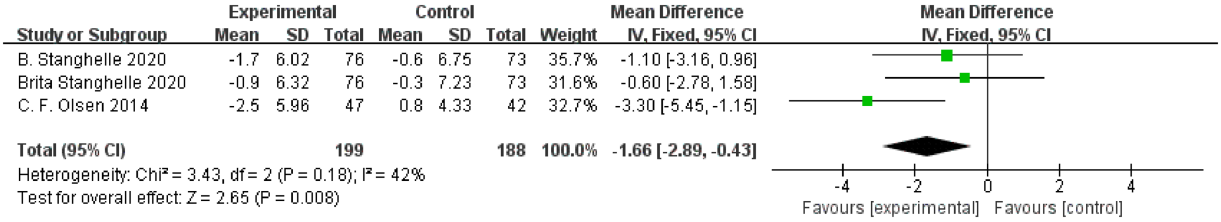

FIGURE 8
Forest plot for the falls efficacy scale international (FES-I).

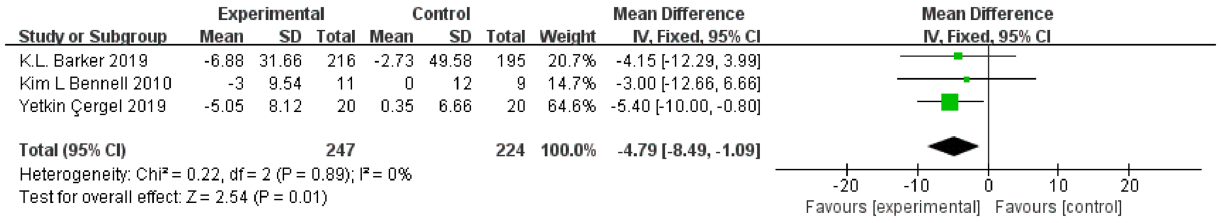

FIGURE 9
Forest plot for kyphosis.

the subgroup analysis. In the under 70 subgroup, comprehensive analysis showed that the exercise group was superior to the control group in QUALEFFO-41 (MD, −9.30, 95% CI, −12.12, −6.48); TUG (MD, −2.28, 95% CI, −3.48, 1.08); and kyphosis (MD, −4.96, 95% CI, −9.11, −0.80). There was no difference in functional reach (MD, −4.56, 95% CI, −14.50, 5.38). In the over 70 subgroup, the comprehensive analysis found that the training group was superior to the control group in QUALEFFO-41 (MD, −2.13 95% CI, −2.58, −1.69); TUG (MD, −0.90, 95% CI, −1.03, 0.77); and functional reach (MD, 2.97, 95% CI, 2.17, 3.70). There was no difference in kyphosis (MD, −4.15, 95% CI, −12.29, 3.99). Subgroup analysis results are shown in Table 2. The forest plots for subgroup analysis are shown in the Supplementary Documents.

Exercise time

We conducted subgroup analysis of the primary outcomes according to exercise time. The included studies were divided into subgroups of under and over 10 weeks of exercise intervention. On the under 10 weeks subgroup analysis, the comprehensive results show that there was no significant difference between the exercise and control groups in the outcomes of functional reach (MD, −4.56, 95% CI, −14.50, 5.38) and VAS (MD, −1.95, 95% CI, −5.55, 1.66). However, the exercise group scored better than controls on the "QUALEFFO-41" (−2.67, 95% CI, −14.60, −5.30). On analysis of the over 10 weeks subgroup, the comprehensive results showed that the exercise group was superior to the control group in "QUALEFFO-41"(MD, −2.67, 95% CI, −4.82, −0.52); functional reach (MD, 2.97, 95% CI, 2.17, 3.76); and VAS (MD, −1.22, 95% CI,-1.64, −0.79). These subgroup analysis results are shown in Table 3. The forest plot for subgroup analysis are shown in the Supplementary material.

Publication bias

We planned to use a funnel chart to evaluate publication bias, but the number of included trials was few ($n = 12$), and the number of patients per trial was also too few (9–216). Thus, we were unable to effectively evaluate publication bias.

Discussion

The main purpose of this study was to evaluate the effects of resistance and balance exercise training on functional status of patients with OVF, through a systematic review and meta-analysis of RCTs. We identified 12 RCTs of patients with OVF using resistance and balance training as the intervention, with functional status and healthy quality of life as the outcomes. For patients with OVF, resistance and balance training ameliorated functional activity, improved body balance, and reduced the degree of back pain. In addition, the positive effect on OVF was seen only when the resistance and balance training lasted for over 10 weeks, and it has little relationship with the patient's age.

The outcome measurements identified in this review were in two primary areas: physical activity and balance capacity. In this meta-analysis, the primary outcomes were scores of QUALEFFO-41, VAS, and functional reach. QUALEFFO-41 assesses quality of life in terms of physical function (17 items), pain (5 items), social function (7 items), mental health (9 items) and general health (3 items) (31). At present, it has become one of the most important indicators to evaluate quality of life in patients with osteoporosis (32). In this meta-analysis, resistance and balance exercise comparatively reduced the QUALEFFO-41 (MD:-3.65, 95% CI, −5.99 to −1.32, $p = 0.002$) in the exercise group, clearly demonstrating the positive significance of the exercise program on quality of life, physical function, and other aspects of OVF patients' lives. On subgroup analysis, the final outcomes had no significant relationship with age or exercise time. Therefore, patients with OVF should actively perform resistance and balance training, and they should perform it for longer than 10 weeks. Evstigneeva et al. (26) found that resistance and balance training not

TABLE 2 Subgroup analysis by age.

Outcome	Age	Included studies	Number	I^2	MD (95% CI)	*p*-value
QUALEFFO-41	<70	3	138	0%	−9.30 (−12.12, −6.48)	$p < 0.00001$
	>70	4	798	51%	−2.13 (−2.58, −1.69)	$p < 0.00001$
TUG	<70	4	268	74%	−2.28 (−3.48, 1.08)	$p = 0.0002$
	>70	2	89	0%	−0.90 (−1.03, 0.77)	$p < 0.00001$
Functional Reach	<70	1	30	0%	−4.56 (−14.50, 5.38)	$p = 0.37$
	>70	5	887	83%	2.97 (2.17, 3.70)	$p < 0.00001$
Kyphosis	<70	2	60	0%	−4.96 (−9.11, −0.80)	$p = 0.02$
	>70	1	411	0%	−4.15 (−12.29, 3.99)	$p = 0.32$

TABLE 3 Subgroup analysis by exercise time.

Outcome	Exercise time	Included studies	Number	I^2	MD (95% CI)	*p*-value
QUALEFFO-41	<10 weeks	1	40	0%	−2.67 (−14.60, −5.30)	$p < 0.0001$
	>10 weeks	6	896	75%	−2.67 (−4.82, −0.52)	$p = 0.02$
Functional Reach	<10 weeks	1	30	0%	−4.56 (−14.50, 5.38)	$p = 0.37$
	>10 weeks	5	887	83%	2.97 (2.17, 3.76)	$p < 0.00001$
VAS	<10 weeks	2	70	97%	−1.95 (−5.55, 1.66)	$p = 0.29$
	>10 weeks	3	154	26%	−1.22 (−1.64, −0.79)	$p < 0.00001$

only significantly reduced the total scores of QUALEFFO-41 but also had an optimistic impact on the individual score of QUALEFFO-41 (26). Functional Reach (FR) is a clinical measurement method to evaluate dynamic balance (33). In this meta-analysis, resistance and balance exercise increased the FR (a better result) (MD:-1.59, 95% CI, −2.61 to −0.58, $p = 0.002$) of the exercise group, clearly demonstrating the dynamic balance ability that exercise programs can create for patients with OVF. The under 70 years and the under 10 weeks subgroups showed no significant differences on subgroup analysis. On subgroup analysis of the over 70 years and over 10 weeks, the exercise group showed superior FR (age MD, 2.97, 95% CI, 2.17, 3.70; exercise time, MD, 2.97, 95% CI, 2.17, 3.70) to that of the control group. Two subgroups were included within the same outcomes. Previously, Watson et al. studied the impact of 8 months of resistance and impact exercise on postmenopausal women with osteoporosis. Compared with scores of the control group (5.4 ± 7.2% versus 0.1 ± 7.2%, $p < 0.001$), the FR was significantly increased in the exercise group (95% CI 3.4% to 7.5% versus−1.8 to 2.1%) (34).This is consistent with our results. Pain was one of the most common clinical symptoms in patients with OVF (35). On this meta-analysis, VAS pain scores (MD, −1.59, 95%CI, −2.61 to −0.58, $p = 0.002$) decreased significantly after resistance and balance training, and the exercise group was significantly better than the controls. On subgroup analysis of under 10 weeks, VAS did not differ in the exercise and control groups. In a subgroup analysis of more than 10 weeks, VAS scores were superior in the exercise group (Exercise time MD, −1.22, 95% CI, −1.64, −0.79) versus the control group. Lyles et al. (35) found that pain led to significantly slower walking speed and greater postural swing. Furthermore, pain reduced muscle strength and increased patients' fear of falling. Therefore, among patients with OVF, pain both increased the fear of falling and decreased physical performance (36). In this study, the secondary outcomes were TUG, kyphosis and FES-I. TUG is a dynamic balance assessment tool that assesses the quality and strength of muscles and has been used to predict repeated falls (37). Increased kyphosis angle is considered to represent the presence of osteoporosis; it also damages balance and postural stability, reduces gait stability, and increases the risk of falls (38). FES-I has been used to assess the fear of falling in daily sports and social activities, and was closely related to physical balance (39). Therefore, secondary outcomes were used to predict fall risk and were closely related to body balance. In this meta-analysis, the secondary outcomes were significantly better in the exercise versus control group (walking speed MD, −1.26, 95% CI, −1.83 to −0.68, $p < 0.0001$; TUG MD, −1.98, 95% CI, −3.25 to −0.71, $p = 0.002$; FES-I MD, −1.66, 95% CI, −2.89 to −0.43, $p = 0.008$; kyphosis MD, −4.79, 95%CI, −8.49 to −1.09, $p = 0.01$). OVF is often accompanied by back pain, hunchback, motor dysfunction, and psychological distress, including anxiety, depression, and fear. Through the above indicators, we found that patients with OVF who performed resistance and balance training experienced significantly reduced pain and fear of falling, improved motor and balance function, and ultimately, may reduce repeat falls risk. The intervention had positive significance for patients' physiological and psychological improvement overall.

Few studies are currently assessing the effects of exercise programs on patients with OVF. Cochrane recently published a review in this area but did not reach a definitive conclusion (40). In the review, Gibbs et al. (40) studied nine randomized controlled trials or semi-randomized trials to evaluate the benefits and hazards of exercise intervention for 4 weeks or more on patients with OVF. They assessed the incidence of re-fracture, pain, falls, health-related quality of life, physical function, and adverse events. They concluded that exercise might improve the patient's physical fitness, but they did not determine the impact of exercise on falls, accidental fractures, or adverse events. This contrasted with our results. One explanation for the divergence

in our results and the data of Gibbs et al. (40) was that the exercise program they investigated was non-specific. The type and intensity of exercise cannot be ignored (40–42). The review by Gibbs et al. (40) involved any type of exercise, including resistance training, balance training, aerobic exercise, tai chi, or other personalized programs. This non-specificity would mean greater results heterogeneity and thus, influence the conclusion. Although there have been some evidence-based recommendations (43, 44) for exercise programs, an optimal program has not yet been determined. The latest clinical prevention and treatment guidelines for osteoporosis strongly recommend that patients carry out a multicomponent program including resistance and balance training and back muscle exercise plans (45). Such an exercise program should enhance muscle strength, improve balance, improve functional status, and ultimately reduce the risk of falls (46, 47). Falling is well known as an important risk factor for fracture (48). For female patients with osteoporosis, falls will increase the risk of spinal fracture 2.5-fold and hip fracture 3.1-fold (49). Therefore, patients with OVF need to perform more resistance and balance exercise; this is consistent with our recommendations.

A resistance and balance training program can improve muscle strength, quality, functional activity, and cardiopulmonary function (50–52) and reduce the risk of falls in older populations (53). Resistance and balance training programs have also positively impacted many diseases, including stroke (54), Parkinson's disease (55), hypertension (56), musculoskeletal pain (57), cardiovascular disease (58), and anxiety symptoms (59). In our review, the intervention program was limited to resistance and balance exercise programs, which reduced the degree of low back pain and improved physical function and quality of life. However, on completing our research, it was clear that there was limited information available regarding resistance and balance motion programs and patients with OVF. To date, there is no specific exercise scheme. Given the complexity of these issues, research conducted within this area appears to lack consistency. Therefore, we explored one specific exercise scheme. In this program, patients were required to complete some warm-up aerobic exercise before the official start. In this movement stage, small and medium-sized muscle groups, and limb joints moved dynamically. The patients began formal exercise after 10 min of warm-up. For this, stood on different surfaces with one leg; the other leg performed various movements to improve their physical balance. The patients could perform chest presses and biceps curls with suitable dumbbells or carry out upright rows for upper back strength and posture. During these activities, the patient actively contracts the scapula and back muscles. This was followed by a series of exercises for enhancing lower extremity muscle strength, e.g., walking backwards, forwards and sideways, climbing steps and performing squats (holding dumbbells or not). Back muscle training developed gradually into five-point, three-point, and one-point support training, progressing from simple to difficult. In this stage, the main muscle groups and joints performed dynamic movements, which enhanced the strength and function of the muscles of the extremities, abdomen, and waist, and improved posture. The program ended with a cool-down period, stretching the muscles of the limbs. In addition to the resistance and balance training, we recommended that patients should exercise for 10 weeks, as soon as possible under the supervision and guidance of medical professionals; this might reduce the probability of falls.

There were limitations to our meta-analysis. The quality of research methods included in this review was variable, but overall, quality was medium to high. Nonetheless, since all studies were observational, there was still the possibility of bias and/or confusion. There were too few studies ($n = 12$) to adequately assess publication bias. On the other hand, due to the small number of studies included, we were only able to conduct subgroup analysis on the primary outcomes. Fortunately, the subgroup analysis supported our conclusions. In addition, the starting time for the resistance and balance training the severity of vertebral fracture (number of vertebral fractures and reduction in body weight) in OVF patients were not clear; this contributed substantially to heterogeneity. In the future, we will seek to identify the correlation of treatment effects with the start time of exercise intervention. It would also be of interest to evaluate outcome measures besides symptom rating scales, such as bone mineral density, cost-effectiveness, or to show heterogeneity of treatment effects.

Conclusion

The resistance and balance exercise training enhanced muscle strength, improved functional activity and balance, reduced pain and fear of falling, which may prevents falls in patients with OVF. For patients of any age with OVF, a resistance and balance exercise training program lasting at least 10 weeks and beginning as early as possible will be beneficial regarding quality of life and activities of daily living. For future research, we will investigate a specific exercise scheme. We will aim to determine the best time for patients to begin the resistance and balance training, and we plan a multicenter, large sample RCT to determine the positive effects of this program.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

XL and ZH conceived the idea for this paper, wrote the protocol, conducted the literature review, and contributed to writing. WC, QC, and FFL extracted the data and edited the manuscript. CC, PL, and FYL conducted research quality assessment. SG and PC conducted a statistical analysis. WY, DL, and SW contributed to writing and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1135063/full#supplementary-material

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Apolipoprotein A1 is associated with osteocalcin and bone mineral density rather than high-density lipoprotein cholesterol in Chinese postmenopausal women with type 2 diabetes mellitus

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Objective: Disturbances in high-density lipoprotein cholesterol (HDL-c) metabolic pathways can affect bone metabolism, which may rely on the particle function of apolipoprotein rather than HDL-c levels. This study aimed to evaluate the correlation of serum HDL-c and apolipoprotein A1 (APOA1) with bone metabolism in Chinese postmenopausal women with type 2 diabetes mellitus (T2DM).

Method: A total of 1,053 participants with complete data were enrolled and separated into three groups based on the HDL-c and APOA1 tertiles. The trained reviewer collected demographic and anthropometric information. Bone turnover markers (BTMs) were determined by standard methods. Bone mineral density (BMD) was measured by dual-energy x-ray absorptiometry.

Results: Overall, the prevalence of osteoporosis was 29.7%. Groups with higher APOA1 have a remarkably more elevated level of osteocalcin (OC), L1-L4 BMD, and *T*-score across the APOA1 tertiles. APOA1 presented a positive correlation with OC (r=0.194, p<0.001), L1-L4 BMD (r=0.165, p<0.001), and *T*-score (r=0.153, p<0.001) rather than HDL-c. Meanwhile, APOA1 remained independently associated with OC (β=0.126, p<0.001), L1-L4 BMD (β=0.181, p<0.001), and *T*-score (β=0.180, p<0.001) after adjustment for confounding factors. APOA1 is also shown to be independently correlated with osteoporosis after adjustment for confounding factors, and the OR (95%CI) was 0.851 (0.784–0.924). In contrast, there was no significant association between HDL-c and osteoporosis. Furthermore, APOA1 seemed to have the largest areas under the curve (AUC) for osteoporosis. The AUC (95% CI) of APOA1 identifying osteoporosis was 0.615 (0.577–0.652). The optimal cut-off value of APOA1 was 0.89g/L (sensitivity: 56.5%, specificity: 67.9%).

Conclusion: APOA1 is independently associated with OC, L1-L4 BMD, and osteoporosis rather than HDL-c in Chinese postmenopausal women with T2DM.

KEYWORDS

osteoporosis, high-density lipoprotein, apolipoprotein A1, bone mineral density, bone turnover markers

Introduction

Bone is a specialized connective tissue with several essential functions that can mechanically support the soft tissues, protect the internal organs and maintain calcium homeostasis. The maintenance of bone mass depends on the balance between bone formation and resorption. This balance ensures that bone can adapt to changes in mechanical loads and minor injuries. The activity of osteoblasts and osteoclasts plays a significant role in this balance. Notably, metabolic dysfunction can easily break bone metabolism homeostasis (1). Type 2 diabetes mellitus (T2DM) is a cluster of metabolic dysfunctions characterized by chronic hyperglycemia. The epidemiological survey showed that T2DM increases the risk of bone fragility fractures, and osteoporosis-related fractures have become major health concerns in T2DM (2–4). T2DM can break the bone metabolism homeostasis and accelerate the development of osteoporosis through some pathways, mainly including elevated advanced glycation end products (5), obesity (6), dyslipidemia (7), increased insulin resistance (8) and chronic inflammatory micro-environment (9) caused by T2DM. Among these mechanisms proposed to explain the association between T2DM and bone homeostasis imbalance, dyslipidemia with dysfunctional high-density lipoprotein cholesterol (HDL-c) involves the development of bone homeostasis imbalance (10).

In addition to the well-recognized anti-atherogenic effects, emerging evidence highlighted that HDL-c and its major protein component of apolipoprotein A1 (APOA1) also play more functional roles in other biological processes, including systemic inflammation, nitric oxide production, oxidative stress, and regulation of bone metabolism homeostasis (11). HDL-c is reported to be closely related to bone physiology and pathology. It was well-recognized that disturbances in lipid metabolic pathways can affect osteoblasts differentiation, leading to bone mass loss (12, 13). Nevertheless, the exact association between HDL-c level and bone mineral density (BMD) generated from epidemiological studies on humans remained uncertain and controversial. Genetic background, age, eating habits, hormonal and metabolic status, and HDL-c functionality relying on the particle function of apolipoprotein rather than HDL-c levels may be responsible for this inconsistency. Likewise, our previous study (14) enrolled 619 postmenopausal women with T2DM revealed that monocyte to APOA1 ratios had a higher area under the curve (AUC) value in identifying osteoporosis than the monocyte to HDL-c ratios, which indicate us that serum APOA1 may be more associated with osteoporosis rather than serum HDL-c. In addition, current epidemiological data on the associations between HDL-c, APOA1 level, and BMD in Chinese postmenopausal with T2DM are lacking. Hence, based on our previous study, we enrolled more participants to further evaluate the associations between HDL-c, APOA1, and BMD, bone turnover markers (BTMs) in Chinese postmenopausal women with T2DM.

Study design and methods

Study design and participants

This cross-sectional study consecutively enrolled postmenopausal women with T2DM admitted to the Department of Endocrinology at the Longyan First Affiliated Hospital of Fujian Medical University between January 2020 and December 2022. Postmenopausal women were defined as participants with twelve consecutive months of amenorrhea. The diagnosis of T2DM was according to the World Health Organization criteria (2019 edition): (1) fasting blood glucose (FBG) $\geq$ 126 mg/dL or 2 h postprandial $\geq$ 200 mg/dL during oral glucose tolerance test or glycosylated hemoglobin A1c (HbA1c) $\geq$ 6.5% or participants with random plasma glucose $\geq$ 200 mg/dL accompanied with classic symptoms of hyperglycemia or hyperglycemic crisis. (2) with diabetic autoimmune antibodies negative and exclude other specific types of diabetes. Participants were excluded if they met the following criteria: (1) history of chronic or acute diseases can lead to secondary bone mass loss (i.e., chronic renal, cardiac, hepatic, thyroid, and rheumatic diseases or bone metastasis). (2) current or prior use of drugs can interfere with bone metabolism (i.e., glucocorticoids, anti-osteoporosis, anti-resorptive or hormonal replacement therapy, calcium or vitamin D supplementation, thiazolidinediones, and urate-lowering therapy). (3) with familial or congenital lipid metabolic disorder. Overall, a total of 1,124 participants were screened. 1,053 participants met the inclusion and exclusion criteria, with complete data in the final analysis. A flowchart describing the selection process of the study population in this study is presented in Figure 1. All procedures were conducted under the Declaration of Helsinki. This study was approved by the ethical committee of Longyan First Affiliated Hospital of Fujian Medical University (LY-2021-072). All participants provided informed consent.

Clinical and biochemical parameters

The trained reviewers used a standard questionnaire to gather demographic data, and they additionally looked over previous medical records. Age, duration of diabetes, current or prior use of medicine, history of the disease, familial or congenital lipid metabolic disorder, smoking, drinking, physical activity, menopausal status, and duration of amenorrhea are all included in the demographic data. Smoking was defined as participants continually or accumulating more than 4 cigarettes a week for at least 6 months according to the guidelines for controlling and monitoring the tobacco epidemic (15). Drinking was defined as participants drinking more than once a year according to the global burden of disease study (16). Participants whose energy expenditure was less than 1.5 metabolic equivalents while awake were considered sedentary (e.g., watching television, reading, writing, or playing video games) (17). The trained research nurses performed anthropometric measurements, including blood pressure (BP), weight, and, height. Body mass index (BMI) was calculated as the weight divided by the square of height (kg/m^2). After resting for more than 5 min, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured on at least three occasions using an electronic sphygmomanometer with an appropriate cuff size. The final BP was determined by taking the average of three readings.

Laboratory assessments were carried out using fasting venous blood samples. After an 8-h overnight fast, blood samples were collected and placed in standardized tubes containing dipotassium ethylenedinitrilo tetra-acetic acid. An auto-biochemical analyzer (Roche Diagnostics Corporation) was used to determine biochemical parameters such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), uric acid (UA), creatinine, fasting blood glucose (FBG), triglycerides (TGs), calcium, and phosphorus. Polyethylene glycol-enhanced immunoturbidimetric test (Maker, Chengdu, China) was utilized to determine ApoA1 concentrations. HbA1c was evaluated by

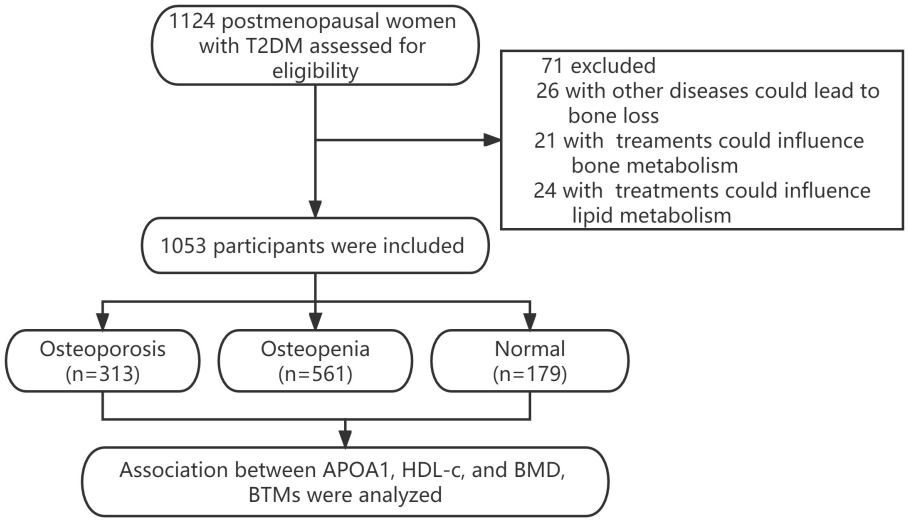

FIGURE 1
Flowchart describing the selection process of the study population in this study.

high-performance liquid chromatography with a D10 set (Bio-RAD). Electro-chemiluminescence immunoassay (Roche Diagnostics GmbH, Germany) was used to measure bone turnover markers such, β-cross-linked C-telopeptide of type I collagen (β-CTX), osteocalcin (OC), intact parathyroid (iPTH), and 25 hydroxyvitamin D (25-OH-D). Serum thyroid stimulating hormone (TSH) levels were also measured to screen for thyroid diseases, which are frequently associated with T2DM and can interfere with bone metabolism. The homeostasis model assessment (HOMA-IR) was calculated using the formula: fasting serum insulin (U/mL) x FBG (mmol/l)/22.5.

Assessment of bone mineral density

The dual-energy X-ray absorptiometry (Hologic, Marlborough, MA, USA) was used to assess the BMD of the participants. During the BMD measurement, participants were in the supine position. The lumbar spine (L1-L4), total hip, and femoral neck make up the BMD measurement areas. The densitometry scanning was performed by experienced radiographers who were also blinded to clinical information. *T*-scores were calculated according to the Hologic densitometry reference value. Longitudinal quality control checks were performed daily using whole-body and L1-L4 lumbar spine phantom provided by the manufacturer. Cross-calibration was performed weekly to monitor variations between the systems. The precision error was 1.0% for the BMD measurement. Postmenopausal women with a T score ≤ -2.5 or a history of bone fragility fractures, $-2.5 < T\text{-score} \leq -1.0$, and *T*-score >-1.0 were considered to have osteoporosis, osteopenia, and normal BDM, respectively.

Statistical analysis

The SPSS 23.0 software (SPSS Inc. IBM) was used to analyze the data. Descriptive and Discrete data are expressed as means ± standard deviation (SD) and frequency tables (N, %), respectively. Participants were divided into three groups based on the tertile of HDL-c and APOA1. A one-way analysis of variance (ANOVA) was used to compare the statistical differences among the groups. Chi-squared ($\chi 2$) test or Fisher exact test was used to compare categorical variables. The Pearson or Spearman correlation analysis was used to evaluate the main correlations between HDL-c, APOA1, and BMD, BTMs. The independent associations between HDL-c, APOA1, and BMD, BTMs was estimated by the multiple regression analysis after adjusting for potential confounding factors. The independent variables of HDL-c and APOA1 for osteoporosis was estimated by the binomial logistic regression analysis after adjusting for other confounding factors. A two-tailed value of $p < 0.05$ was considered statistically significant.

Results

Clinical and laboratory characteristics based on tertiles of HDL-c and APOA1

A total of 1,053 postmenopausal women with T2DM were included in this study. The mean age of participants was 56.4 ± 6.2 years, and the mean menopausal duration was 5.2 ± 2.2 years. The hypoglycemic agents type of participants used were presented in Supplementary Table 1. The clinical and laboratory characteristics of participants based on tertiles of HDL-c and APOA1 were summarized in Table 1. The ANOVA analysis showed no significant differences in age, diabetic duration, menopausal duration, HbA1c, TC, LDL-c, creatinine, ALT, and the proportion of sedentary behavior, smoking, and drinking across the HDL-c and APOA1 tertiles ($p > 0.05$). Meanwhile, groups with higher HDL-c and APOA1 have remarkably lower BMI, TG, UA, and HOMA-IR levels and a lower prevalence of hypertension ($p < 0.05$).

TABLE 1 Clinical and laboratory characteristics of participants based on tertiles of HDL-c and APOA1.

Variable	Tertiles of HDL-c			*p*	Tertiles of APOA1			*p*
	T1	T2	T3		T1	T2	T3	
Age (year)	56.7 ± 6.3	55.8 ± 6.6	56.7 ± 5.7	0.102	56.6 ± 6.7	56.1 ± 6.5	56.7 ± 5.2	0.348
Duration (year)	8.1 ± 2.5	8.2 ± 2.2	8.1 ± 2.7	0.603	8.2 ± 2.7	8.1 ± 2.1	8.1 ± 2.2	0.716
BMI (kg/m^2)	25.9 ± 2.8^{ab}	24.2 ± 2.3^{ac}	22.4 ± 2.5^{bc}	<0.001	25.6 ± 2.9^{ab}	24.4 ± 2.5^{ac}	22.1 ± 3.3^{bc}	<0.001
HbA1c (%)	9.1 ± 4.5	8.9 ± 1.4	8.9 ± 1.4	0.520	9.0 ± 1.5	9.1 ± 1.2	8.9 ± 1.3	0.532
TG (mmol/L)	3.4 ± 2.3^{ab}	2.0 ± 0.9^{ac}	1.2 ± 0.7^{bc}	<0.001	2.7 ± 1.6^{ab}	2.2 ± 1.3^{ac}	1.8 ± 1.2^{bc}	<0.001
TC (mmol/L)	5.1 ± 1.2	5.0 ± 1.2	5.1 ± 1.1	0.408	5.2 ± 1.2^{b}	5.1 ± 1.1	4.9 ± 1.1^{b}	0.088
HDL-c (mmol/L)	0.84 ± 0.09^{ab}	1.02 ± 0.07^{ac}	1.35 ± 0.26^{bc}	<0.001	0.96 ± 0.22^{ab}	1.08 ± 0.21^{ac}	1.18 ± 0.25^{bc}	<0.001
LDL-c (mmol/L)	3.5 ± 1.0	3.6 ± 0.9	3.5 ± 1.0	0.428	3.6 ± 1.0	3.6 ± 1.0	3.5 ± 0.9	0.326
APOA1 (g/L)	0.90 ± 0.19^{ab}	0.97 ± 0.20^{ac}	1.05 ± 0.26^{bc}	<0.001	0.76 ± 0.07^{ab}	0.95 ± 0.05^{ac}	1.21 ± 0.20^{bc}	<0.001
UA (umol/L)	390 ± 94^{ab}	365 ± 72^{ac}	300 ± 70^{bc}	<0.001	368 ± 78^{ab}	342 ± 83^{ac}	323 ± 98^{bc}	<0.001
Creatinine (umol/L)	70.0 ± 13.9	68.3 ± 12.8	69.4 ± 13.2	0.229	68.6 ± 13.4	69.6 ± 13.7	69.5 ± 12.7	0.534
ALT (IU/L)	33.2 ± 9.2	33.7 ± 9.3	32.9 ± 10.6	0.554	33.9 ± 10.4	33.1 ± 9.6	32.9 ± 9.1	0.346
HOMA-IR	4.6 ± 2.1^{ab}	4.0 ± 1.8^{ac}	2.6 ± 1.9^{bc}	<0.001	4.2 ± 2.2^{ab}	3.4 ± 1.9^{a}	3.6 ± 2.0^{b}	<0.001
Menopausal duration (year)	5.2 ± 1.9	5.3 ± 2.1	5.1 ± 2.2	0.678	5.2 ± 2.1	5.1 ± 2.4	5.3 ± 1.7	0.716
Hypertension, *n* (%)	190 (55.9)^{ab}	151 (43.5)^{ac}	81 (22.4)^{bc}	<0.001	188 (53.0)^{ab}	144 (41.0)^{ac}	94 (27.1)^{bc}	<0.001
Smoking, *n* (%)	10 (2.9)	9 (2.6)	6 (1.7)	0.515	8 (2.3)	12 (3.4)	5 (1.4)	0.226
Drinking, *n* (%)	56 (16.5)	55 (15.7)	57 (15.7)	0.951	57 (16.1)	61 (17.4)	50 (14.4)	0.562
Sedentary behavior, *n* (%)	100 (29.4)	97 (27.6)	92 (25.4)	0.493	97 (27.3)	95 (27.1)	97 (28.0)	0.964

BMI, body mass index; HbA1c, Glycated hemoglobin; UA, uric acid; TG, triglyceride; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, Low density lipoprotein cholesterol; APOA1, Apolipoprotein A1; HOMR-IR, Homeostasis model assessment insulin resistance.
[a]$p < 0.05$: T1 vs T2.
[b]$p < 0.05$: T1 vs T3.
[c]$p < 0.05$: T2 vs T3.

BTMs and BMD of participants based on tertiles of HDL-c and APOA1

The prevalence of osteoporosis was 29.7% in postmenopausal with T2DM. The mean BMD was 0.89 ± 0.11 g/cm3 in L1-L4, 0.81 ± 0.09 g/cm3 in the hip, and 0.70 ± 0.12 g/cm3 in the femoral neck. The BTMs and BMD of participants based on tertiles of HDL-c and APOA1 were summarized in Table 2. The results showed no significant differences in β-CTX, 25-OH-D, iPTH, ALP, calcium and phosphorous levels, hip BMD and *T*-score, femoral neck BMD, and *T*-score across the HDL-c and APOA1 tertiles ($p > 0.05$). In addition, there were also no significant differences in levels of OC, L1-L4 BMD, and *T*-score across the HDL-c tertiles ($p > 0.05$). In contrast, groups with higher APOA1 have a remarkably more elevated level of OC, L1-L4 BMD, and *T*-score across the APOA1 tertiles ($p < 0.05$). The prevalence of osteoporosis among different tertiles of HDL-c and APOA1 is illustrated in Figure 2. The results displayed a decreasing trend in the prevalence of osteoporosis across the HDL-c and APOA1 tertiles ($p < 0.05$).

Main correlations of HDL-c and APOA1 with BTMs and BMD

The main correlations of HDL-c and APOA1 with BTMs and BMD are presented in Table 3. The Pearson or Spearman correlation analysis revealed that HDL-c and APOA1 were not significantly correlated with β-CTX, 25-OH-D, iPTH, ALP, calcium and phosphorous, hip BMD and *T*-score, femoral neck BMD and *T*-score ($p > 0.05$). In addition, HDL-c was also not significantly correlated with OC, L1-L4 BMD, and *T*-score ($p > 0.05$). Nevertheless, APOA1 presented a positive correlation with OC ($r = 0.194$, $p < 0.001$), L1-L4 BMD ($r = 0.165$, $p < 0.001$), and *T*-score ($r = 0.153$, $p < 0.001$).

Impact of HDL-c and APOA1 on OC, L1-L4 BMD, and *T*-score

The multiple linear regression analysis was also conducted to further evaluate the associations between HDL-c and APOA1 on OC, L1-L4 BMD, and *T*-score. As shown in Table 4, after adjustment for age, BMI, diabetic duration, menopausal duration, hypertension, sedentary behavior, smoking, drinking (Model 1), HbA1c, TG, HDL-c, APOA1, LDL-c, creatinine, ALT, UA, and HOMA-IR (Model 2), APOA1 was shown to be positively correlated with OC, L1-L4 BMD, and *T*-score. APOA1 remained independently associated with OC ($\beta = 0.126$, $p < 0.001$), L1-L4 BMD ($\beta = 0.181$, $p < 0.001$), and *T*-score ($\beta = 0.180$, $p < 0.001$) after additional adjustment for BTMs like β-CTX, 25-OH-D, ALP, iPTH, calcium, phosphorous (Model 3), and OC (Model 4). Furthermore, there was no significant association of HDL-c with L1-L4 BMD, L1-L4 *T*-score, and OC after adjustment for Model 1, Model 2, and Model 4.

TABLE 2 BTMs and BMD of participants based on tertiles of HDL-c and APOA1.

Variable	Tertiles of HDL-c			*p*	Tertiles of APOA1			*p*
	T1	T2	T3		T1	T2	T3	
OC (ng/mL)	16.6 ± 3.7	16.9 ± 3.3	16.4 ± 3.0	0.209	15.7 ± 3.4[ab]	16.6 ± 3.1[ac]	17.6 ± 3.3[bc]	<0.001
β-CTX (ng/mL)	0.49 ± 0.18	0.47 ± 0.17	0.47 ± 0.24	0.491	0.48 ± 0.21	0.48 ± 0.21	0.47 ± 0.17	0.879
25-OH-D (nmol/L)	49.4 ± 22.5	48.1 ± 20.5	50.9 ± 24.5	0.240	50.3 ± 25.3	48.9 ± 21.9	49.2 ± 20.4	0.664
iPTH (ng/L)	36.0 ± 13.3[a]	33.8 ± 13.2[a]	34.8 ± 14.4	0.101	35.3 ± 15.1	35.0 ± 14.3	34.3 ± 11.1	0.538
ALP (IU/L)	77.3 ± 20.3	79.7 ± 20.3	78.6 ± 19.6	0.184	79.4 ± 21.1	78.9 ± 20.6	77.1 ± 19.1	0.289
Calcium (mmol/L)	2.31 ± 0.11	2.31 ± 0.11	2.32 ± 0.11	0.366	2.31 ± 0.10	2.32 ± 0.10	2.32 ± 0.11	0.562
Phosphorous (mmol/L)	1.22 ± 0.17	1.21 ± 0.15	1.22 ± 0.19	0.272	1.23 ± 0.17	1.21 ± 0.18	1.23 ± 0.17	0.169
L1-L4 BMD (g/cm^3)	0.89 ± 0.11	0.89 ± 0.10	0.89 ± 0.12	0.703	0.86 ± 0.10[ab]	0.89 ± 0.09[ac]	0.92 ± 0.13[bc]	<0.001
L1-L4 *T*-score	−1.8 ± 1.0[b]	−1.7 ± 0.8	−1.6 ± 0.9[b]	0.028	−1.9 ± 0.8[ab]	−1.7 ± 0.8[ac]	−1.4 ± 1.2[bc]	<0.001
Hip BMD (g/cm^3)	0.81 ± 0.06	0.82 ± 0.11	0.81 ± 0.10	0.642	0.82 ± 0.09	0.81 ± 0.11	0.81 ± 0.12	0.576
Hip *T*-score	−1.4 ± 0.6	−1.3 ± 0.7	−1.4 ± 0.8	0.617	−1.3 ± 0.9	−1.4 ± 0.5	−1.4 ± 0.7	0.594
Femoral neck BMD (g/cm^3)	0.70 ± 0.13	0.71 ± 0.08	0.71 ± 0.12	0.492	0.71 ± 0.13	0.71 ± 0.10	0.70 ± 0.11	0.376
Femoral neck *T*-score	−1.3 ± 0.9	−1.2 ± 0.8	−1.2 ± 0.7	0.526	−1.2 ± 0.7	−1.2 ± 0.6	−1.3 ± 0.9	0.488

OC: osteocalcin. β-CTX: β-cross-linked C-telopeptide of type I collagen (β-CTX). 25-OH-D: 25 hydroxyvitamin D. iPTH: intact parathyroid hormone. ALP: alkaline phosphatase. BMD: bone mineral density. BTMs: bone turnover markers.

[a]$p < 0.05$: T1 vs T2.

[b]$p < 0.05$: T1 vs T3.

[c]$p < 0.05$: T2 vsT3.

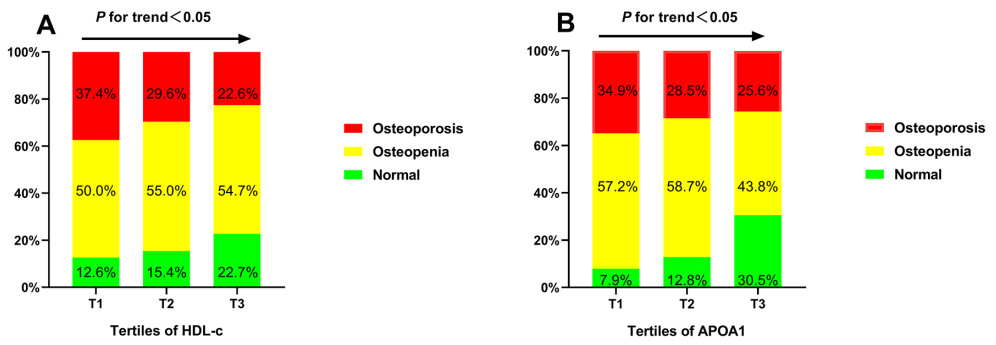

FIGURE 2
The prevalence of osteoporosis, osteopenia, and normal BMD across the HDL-c **(A)** and APOA1 **(B)** tertiles.

Impact of HDL-c and APOA1 on osteoporosis

The binomial logistic regression analysis was also conducted to assess the independent variables of HDL-c and APOA1 for osteoporosis. As shown in Figure 3, the results showed that the risk of osteoporosis significantly decreased with the increase of APOA1. After adjustment for age, BMI, diabetic duration, menopausal duration, hypertension, sedentary behavior, smoking, and drinking (model 1), APOA1 was independently correlated with osteoporosis, and the ORs (95%CI) was 0.889 (0.832–0.950). A significant association between osteoporosis and APOA1 was also observed after additional adjustment for HbA1c, TG, HDL-c, APOA1, LDL-c, creatinine, ALT, UA, and HOMA-IR (model 2), and the ORs (95%CI) was 0.843 (0.782–0.910) respectively. Furthermore, the ORs remained significant after further adjustment for bone turnover markers like OC, β-CTX, 25-OH-D, iPTH, ALP, calcium, and phosphorous (model 3), and the ORs (95%CI) was 0.851 (0.784–0.924). In contrast, there was no significant association between HDL-c and osteoporosis in any models.

Values of HDL-c and APOA1 in identifying osteoporosis

Figure 4 shows the performance for evaluating the value of different serum lipids for osteoporosis risk. The results showed that the AUC of APOA1 was larger than other serum lipids ($p < 0.05$). The AUC (95% CI) of APOA1 in identifying osteoporosis was 0.615 (0.577–0.652). The optimal cut-off value of APOA1 was 0.89 g/L, with a sensitivity of 56.5% and specificity of 67.9% (Table 5).

TABLE 3 Main correlations of HDL-c and APOA1 with BTMs and BMD.

Variable	HDL-c		APOA1	
	R	*p*	*R*	*p*
OC (ng/mL)	−0.002	0.948	0.194	<0.001
β-CTX (ng/mL)	0.004	0.884	−0.027	0.390
25-OH-D (nmol/L)	0.033	0.287	−0.009	0.758
iPTH (ng/L)	−0.046	0.132	−0.032	0.304
ALP (IU/L)	−0.056	0.070	−0.049	0.114
Calcium (mmol/L)	0.009	0.758	0.012	0.692
Phosphorous (mmol/L)	0.019	0.537	0.038	0.217
L1-L4 BMD (g/cm^3)	−0.018	0.555	0.165	<0.001
L1-L4 *T*-score	0.044	0.154	0.153	<0.001
Hip BMD (g/cm^3)	0.021	0.488	−0.005	0.879
Hip *T*-score	0.010	0.746	−0.009	0.758
Femoral neck BMD (g/cm^3)	0.017	0.583	−0.020	0.528
Femoral neck *T*-score	0.006	0.839	−0.021	0.498

OC: osteocalcin. β-CTX: β-cross-linked C-telopeptide of type I collagen (β-CTX). 25-OH-D: 25 hydroxyvitamin D. iPTH: intact parathyroid hormone. ALP: alkaline phosphatase. BMD: bone mineral density. BTMs: bone turnover markers.

TABLE 4 Multivariate linear regression analysis between HDL-c, APOA1 and OC, L1-L4 BMD, LI-L4 *T*-score.

Variable	HDL-c		APOA1	
	β	*p*	*β*	*p*
OC (ng/mL)				
Model 1	−0.059	0.102	0.186	<0.001
Model 2	−0.058	0.155	0.123	<0.001
Model 3	−0.047	0.243	0.126	<0.001
L1-L4 BMD (g/cm^3)				
Model 1	0.015	0.690	0.171	<0.001
Model 2	0.066	0.120	0.195	<0.001
Model 4	0.078	0.078	0.181	<0.001
L1-L4 *T*-score				
Model 1	0.050	0.169	0.180	<0.001
Model 2	0.084	0.053	0.202	<0.001
Model 4	0.082	0.068	0.180	<0.001

Model 1: Adjusted for age, BMI, diabetic duration, menopausal duration, hypertension, sedentary behavior, smoking and drinking. Model 2: Additional adjustment for HbA1c, TG, HDL-c, APOA1, LDL-c, creatinine, ALT,UA and HOMA-IR based on Model 1. Model 3: Additional adjustment for β-CTX, 25-OH-D, iPTH, ALP, calcium, and phosphorous based on Model 2. Model 4: Additional adjustment for OC based on Model 3.

Discussion

Emerging evidence demonstrated that disturbances in lipid metabolic pathways could affect osteoblasts differentiation, leading to bone mass loss. Nevertheless, the association between HDL-c, APOA1, and BMD, BTMs in Chinese postmenopausal women with T2DM remained uncertain. This cross-sectional study revealed that APOA1 positively correlates with OC, L1-L4 BMD, and *T*-score after adjusting for potential confounding factors. In addition, APOA1 is also shown to be independently associated with lower odds of osteoporosis. In contrast, there was no significant association between HDL-c and BMD, BTMs. Furthermore, APOA1 has a more considerable osteoporosis-identifying value than other serum lipids.

Osteoporosis and T2DM are prevalent diseases that have become major public health concerns in the increasingly aging population. Epidemiological studies reported that the prevalence of osteoporosis is approximately 30 to 40% in Chinese postmenopausal with T2DM (18). The prevalence of osteoporosis is 29.4% in our study, which is consistent with the previous studies. Lipid metabolites are widely distributed throughout the human body and are essential in several metabolic pathways. T2DM is a kind of metabolic disorder that is accompanied by dyslipidemia. The main manifestations of lipid changes in T2DM are reduced HDL-c and elevated LDL-c. Besides the well-recognized anti-atherogenic effects, recent advances in bone metabolism and lipid highlighted that HDL-c plays a functional role in bone metabolism. The changes in HDL-c can lead to significant disruptions in the bone microenvironment. Reduced HDL levels have been associated with developing an inflammatory micro-environment (19). It is well-recognized that chronic inflammation strongly affects bone remodeling, affects the function of osteoblasts and osteoclast functions to varying degrees, and therefore plays a central role in developing bone-related metabolic pathology (20, 21). Triantaphyllidou et al. found a novel function of HDL in the pathogenesis of degenerative and metabolic bone diseases. Perturbations in the HDL metabolic pathway predispose to bone mass loss by inhibiting osteoblasts differentiation and modification of specific bone-associated chemokines and signaling cascades (12). Elevated bone marrow adiposity can affect osteocytes to varying degrees and is involved in the pathogenesis of bone-related pathologies, such as osteoporosis. Novel data from experimental mice or cells suggest that dysfunctional HDL status can influence the bone marrow microenvironment and the osteoblastic niche (22, 23). These findings may indicate that low and dysfunctional HDL may lead to reduced bone mass and impaired bone quality, resulting in an increased prevalence of osteoporosis.

Despite most studies confirming that dysfunctional HDL-c can directly affect bone metabolic homeostasis in mice or *in vivo*, the exact association between HDL-c level and BMD in real-world studies is inconsistent. In Asian postmenopausal women, two cross-sectional studies that enrolled more than 1,000 Korean women suggested a positive association between HDL-c and lumbar spine BMD in postmenopausal women (24, 25). In contrast, no association was found between HDL-c and BMD in another study that enrolled 355 postmenopausal Korean women (26). In western postmenopausal women, a cohort study that enrolled more than 3,000 Swedish postmenopausal women showed that HDL-c was negatively correlated with wrist BMD (27). Nevertheless, two cohort studies enrolled American and Danish postmenopausal women did not find an association between HDL-c and BMD (28, 29). There was also no significant association between HDL-c and BMD in Chinese postmenopausal women in two cohort studies that enrolled more than 2000 postmenopausal women (30, 31). Genetic background, age, eating habits, and hormonal and metabolic status may be responsible for this inconsistency. There is a lack of epidemiological data on the correlation between HDL-c, APOA1 levels, and BMD in Chinese postmenopausal women. In our study, after adjusting for several potential confounding factors, there was no significant association

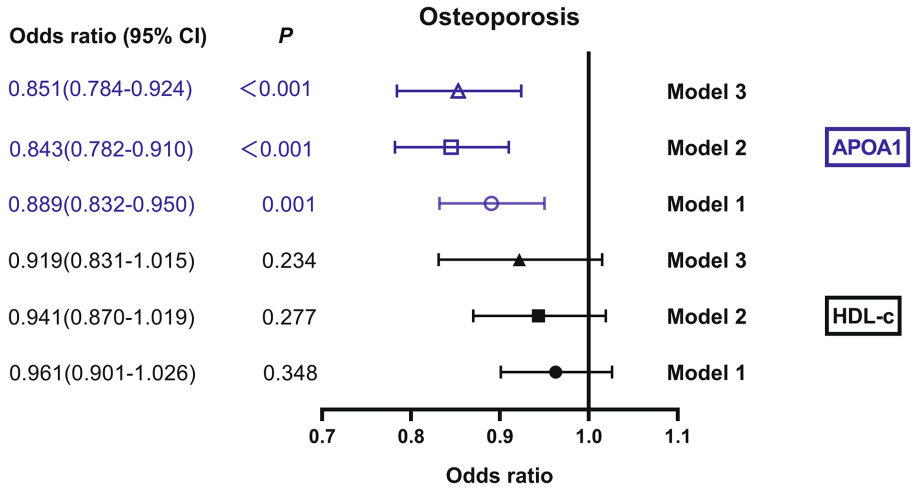

FIGURE 3
Impact of HDL-c and APOA1 on osteoporosis by binomial logistic regression analysis. Model 1: adjusted for age, BMI, diabetic duration, menopausal duration, hypertension, sedentary behavior, smoking, and drinking. Model 2: additional adjustment for HbA1c, TG, HDL-c, APOA1, LDL-c, creatinine, ALT, UA, and HOMA-IR. Model 3: further adjustment for OC, β-CTX, 25-OH-D, iPTH, ALP, calcium, and phosphorous.

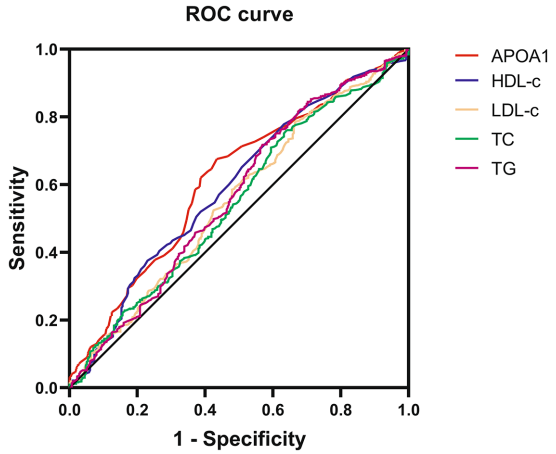

FIGURE 4
Receiver operating characteristic curves for the cutoff value of serum lipids identifying osteoporosis.

between HDL-c and BMD, BTMs. This finding is consistent with the above two studies involving Chinese postmenopausal women. It is worth noting that HDL-c functionality relies on the particle function of HDL apolipoprotein and lipid content rather than HDL levels.

APOA1 is the main protein component of HDL-c that plays a crucial role in the biogenesis and functions of HDL-c. Besides the well-documented cardioprotective functions, APOA1 is also identified to have additional beneficial functions like anti-inflammatory, anti-atherogenic, anti-apoptotic, and anti-thrombotic (32). Recent data from experimental mice also confirmed that ApoA1 deficiency generates changes in the bone cell precursor population, can increase adipoblast and decreases osteoblast production (13). In addition, APOA1 deficiency can also influence bone metabolism by reshaping bone marrow adipocyte phenotypic and molecular characteristics (33, 34). OC is a product of osteoblasts that accumulates in the extracellular matrix of bone. Serum OC level can reflect the osteoblast activity and the rate of bone formation. Our study showed that APOA1 is positively associated with OC after adjustment for potential confounding factors. This finding may suggest that serum APOA1 level can reflect the osteoblasts' activity. Furthermore, APOA1 is also shown to be independently correlated with L1-L4 BMD and lower odds of osteoporosis. These findings may indicate that APOA1 has an anti-osteoporosis effect by regulating the activity of osteoblasts. Meanwhile, there remains a question of why APOA1 is only associated with L1-L4 BMD. More studies are needed to confirm these findings and illustrate the potential mechanisms.

Strength and limitation

This study adjusted several potential confounding variables in the final analysis and included enough more than 1,000 participants can represent the population of Chinese postmenopausal women with T2DM. Several limitations are needed to mention in our study. First, this study was designed as a cross-sectional study that cannot directly reflect the associations between HDL-c, APOA1 and, BTMs, BMD. Second, the study population is Chinese postmenopausal women with T2DM, and these associations may not apply to other races, hormonal status, and metabolic status. Third, APOA1 seemed to have a more significant identifying value of osteoporosis than other serum lipids, and more studies with enough follow-up to confirm these findings.

Conclusion

Work completed on data showed that the associations between HDL-c, APOA1 level, and BMD in Chinese postmenopausal with T2DM remained uncertain. Our study showed that serum APOA1 is positively associated with OC, L1-L4 BMD, and *T*-score rather than HDL-c. Furthermore, APOA1 is also shown to be independently associated with lower odds of osteoporosis. These findings may

TABLE 5 ROC curve analysis of serum lipids in identifying osteoporosis.

Variables	AUC(95% CI)	Cut-Off value	Sensitivity (%)	Specificity (%)
APOA1	0.615 (0.577–0.652)	0.89	56.5	67.6
HDL-c	0.584 (0.546–0.622)	1.15	77.0	37.6
LDL-c	0.555 (0.516–0.593)	2.86	33.5	78.5
TC	0.548 (0.510–0.587)	4.32	36.1	75.9
TG	0.567 (0.527–0.605)	2.37	56.9	28.2

TG, triglyceride; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; APOA1, Apolipoprotein A1.

indicate that APOA1 has an anti-osteoporosis effect by regulating the activity of osteoblasts. At the same time, more studies are needed to confirm these findings further and illustrate the potential mechanisms.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethical Committee of Longyan First Affiliated Hospital of Fujian Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

WW took charge of the software and contributed to writing—the original draft. WW, ZC, FL, XG, and MT conducted the investigation. XG and MT contributed to data curation and writing editing. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1182866/full#supplementary-material

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Association between bone mineral density and cardiovascular disease in older adults

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Background and aims: Cardiovascular disease and osteoporosis are common diseases in older adults with high morbidity. The study on the interaction between the two in pathogenic mechanisms has been paid much attention by the majority of researchers. This study aimed to explore the relationship between bone mineral density and cardiovascular disease in older adults.

Methods: The primary data was downloaded from the National Health and Nutrition Examination Survey database of the United States. Multivariate logistic regression model, generalized additive model, and smooth curve fitting were used to explore the relationship between bone mineral density and cardiovascular events risk. When a curve relationship was found, a two-piecewise linear model was used to calculate the inflection point. In addition, subgroup analysis was also performed.

Results: A total of 2097 subjects were included in this study. After adjusting for potential confounders, no significant association was found between lumbar bone mineral density and cardiovascular disease, while femur bone mineral density had a non-linear relationship with cardiovascular disease, with an inflection point of 0.741 gm/cm^2. When bone mineral density was <0.741 gm/cm^2, the risk of cardiovascular disease decreased speedily. Once bone mineral density exceeded this value, the risk of cardiovascular disease continued to decrease, but the trend became significantly slower. Compared with patients with normal bone mass, osteoporosis was associated with a 2.05-fold increased risk of cardiovascular disease (95% CI 1.68–5.52). There were no significant differences in interaction tests of all subgroups (p for interaction >0.05) except race.

Conclusion: Our results indicated that bone mineral density was closely associated with the prevalence of cardiovascular disease in older adults over 60 years old, especially the femur bone mineral density was negatively non-linear associated with cardiovascular disease risk, with an inflection point of 0.741 gm/cm^2.

KEYWORDS

bone mineral density, osteoporosis, cardiovascular disease, older adults, risk factor

Introduction

Cardiovascular disease (CVD) ranks first among chronic non-communicable diseases in the world. It is characterized by high morbidity, disability, and mortality, seriously affecting the quality of life (1). According to the latest annual statistics of the American College of Cardiology, the overall prevalence of CVD in the adult population was 9.3% (26.1 million), and about

874,613 people died of CVD in 2019 (2). In Asia, a meta-analysis found that the probability of fatal cardiovascular events in a population free of CVD history at baseline was 3.68/per 1,000 person-years (3). With the acceleration of the population aging process, CVD will bring a greater social and economic burden, and how to effectively prevent and treat CVD is a huge challenge.

Osteoporosis is a multifactorial metabolic bone disease characterized by decreased bone mass and destruction of bone microstructure, resulting in increased bone fragility and fracture risk. The fundamental mechanism is the imbalance of bone homeostasis maintained by bone formation and bone destruction (4). The prevalence of osteoporosis in the world's older adults was 21.7%, with the highest prevalence of 24.3% in Asian countries, followed by Europe (16.7%) and the United States (11.5%) (5). Dual-energy X-ray absorptiometry (DXA) is the most widely used diagnostic method of osteoporosis. The classification criteria for DXA measurement released by the World Health Organization were: normal bone mass (T score ≥ -1); osteopenia (T score > -2.5, and < -1); osteoporosis (T score ≤ -2.5); severe osteoporosis (T score ≤ -2.5, and accompanied with brittle fractures) (6, 7).

Recent animal experiments showed that the femur and lumbar bone mineral density (BMD) decreased by 6.9 and 3.5%, respectively, in the myocardial infarction mouse model established by artificial ligation of the left anterior descending artery (8). In addition, several populations' clinical studies had reported a possible association between BMD and CVD occurrence. For example, Wiklund et al. found that lower BMD was associated with an increased risk of myocardial infarction in both men and women (9). Other epidemiological studies have reported an association between reduced BMD and higher morbidity and mortality in stroke (10) and heart failure (11).

In the present study, we conducted multivariate logistic regression and stratified analysis to explore the possible relationship between BMD and the risk of CVD in older adults over 60 years old. This study is expected to provide more guidance on early monitoring and clinical prevention.

Methods

The raw data used in this study came from the National Health and Nutrition Examination Survey (NHANES) of the United States.[1] NHANES is a nationwide cross-sectional study based on diverse levels of population. It integrates the demographics, dietary, examination, laboratory, questionnaire, and limited access data. This information will be used to evaluate the residents' nutritional status and its association with disease prevention. In this study, we extracted the population data aged >60 years from 2005–2010, 2013–2014, and 2017–2018, to increase the sample size and improve statistical efficiency (BMD measurements in 2011–2012 and 2015–2016 were limited to people aged 8–59 years, so they were excluded). Detailed study design proposals were available on the NHANES website. In addition, we excluded patients with malignancy and thyroid disease (1926, 1,195, respectively). Subjects without BMD measurements (3,467 cases) and uncertain history of CVD (21 cases) were removed, too. 472 participants with missing other baseline data were also excluded and the remaining 2097 entered the final analysis. Detailed screening criteria for the study were provided in Figure 1. The NCHS Research Ethics Review Board (ERB) approved all agreements and each participant signed a written informed consent form.

CVD was defined as a range of self-reported diseases, including congestive heart failure, coronary heart disease, angina pectoris, and heart attack. These questions were described in the NHANES questionnaire dataset as MCQ160 b-e (has a doctor or other health professional ever told you that you had congestive heart failure, coronary heart disease, angina/angina, or heart attack?). If all the above-mentioned diseases were denied, the subject was considered to have no CVD. On the contrary, if one or more of the diseases were identified, the subject was considered to have developed CVD (12).

DXA is the most widely used method of BMD measurement in clinical practice, which has the advantages of fast speed, ease to use, and low radiation exposure. The lumbar and femur were scanned using the Hologic Discovery model A densitometer (Hologic, Inc., Bedford, Massachusetts). BMD measurements were performed by trained and certified radiologists, and those who were pregnant, had used contrast material in the last 7 days, and were overweight than required were excluded. Detailed information about BMD measurements and procedures can be found on the NHANES website.

Covariables include age, sex, race, systolic blood pressure, diastolic blood pressure, body mass index, waist circumference, hypertension, hyperlipidemia, diabetes mellitus, chronic kidney disease, liver disease, smoke, albumin, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, phosphorus, calcium, high-density lipoprotein cholesterol, total cholesterol, glycated hemoglobin, and Vitamin D. Relevant medical history can be found in the corresponding column in the questionnaire data. Hypertension was defined as a self-reported history of hypertension (BPQ020 Have you ever been told by a doctor or other health professional that you had hypertension, answered yes), or systolic blood pressure ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg. Hyperlipidemia was defined as a self-reported history of hyperlipidemia (BPQ080 Have you ever been told by a doctor or other health professional your blood cholesterol level was high, answered yes), or blood cholesterol concentration ≥ 5.7 mmol/L. Diabetes was defined as a self-reported history of diabetes (DIQ010 Have you ever been told by a doctor or other health professional that you had diabetes other than during pregnancy, answered yes), or glycated hemoglobin $\geq 6.5\%$. Chronic kidney disease was defined as a self-reported history of kidney disease (KIQ022 Have you ever been told by a doctor or other health professional that you had a weak or failing kidney, do not include kidney stones, bladder infections, or incontinence, answered yes), or creatinine ≥ 177 mmol/L. Liver disease was defined as a self-reported history of liver disease (MCQ160l has a doctor or other health professional ever told you that you had any kind of liver condition, answered yes), median liver stiffness ≥ 7.3 kPa or median controlled attenuated parameter ≥ 240 dB/m measured by liver ultrasound transient elastography (13). Smoking status was determined by serum cotinine concentration (≥ 10 ng/mL was defined as a smoker, and < 10 ng/mL was a non-smoker) (14).

1 https://www.cdc.gov/nchs/nhanes/index.htm

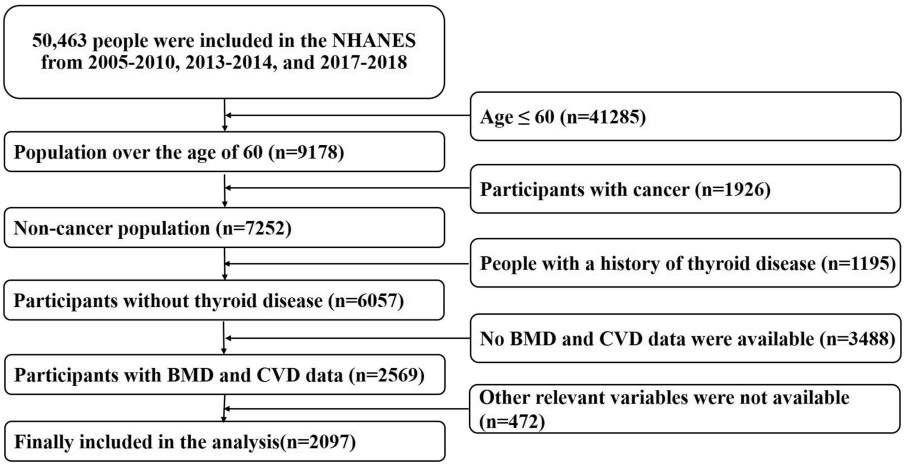

FIGURE 1
Screening criteria for the study population.

All statistical analyses were calculated using the R package, version 4.2.0,[2] and EmpowerStats software.[3] $p < 0.05$ indicated that the difference was statistically significant. Continuous variables were expressed as mean ± SD or median (interquartile range, IQR), while categorical variables were presented as percentages (%). Multivariable logistic regression models were performed to explore the relationship between BMD and CVD occurrence. After adjusting for confounding factors of CVD, a generalized additive model and smooth curve fitting were used to achieve visualization. When the nonlinear relationship was found, a two-piecewise linear regression model was used to analyze. Then, subgroup analyses were used to find the heterogeneity between different groups stratified by age, sex, race, body mass index, hypertension, hyperlipidemia, diabetes mellitus, chronic kidney disease, liver disease, and smoking status.

Results

A total of 2097 people were included in this study. The description of baseline characteristics was shown in Table 1. 347 people suffered from CVD, with an incidence rate of 16.55%. The average age of the population was 68.92 ± 6.37, with males accounting for 51.88% and females for 48.12%. Non-Hispanic whites accounted for the highest proportion among different ethnic groups at 45.45%. Compared with the no CVD group, participants in the CVD group tended to be older, more male, more smokers, and have higher rates of hypertension, hyperlipidemia, diabetes, and chronic kidney disease. In addition, among different groups of diastolic blood pressure, body mass index, waist circumference, albumin, blood urea nitrogen, creatinine, calcium, high-density lipoprotein cholesterol, total cholesterol, glycosylated hemoglobin, vitamin D, and lumbar BMD, CVD occurrence was significantly different, whereas others were not.

As shown in Table 2, three multivariate logistic regression models were constructed to investigate the association between CVD and BMD of the lumbar and femur respectively: crude model, without covariate adjustment; model 1, adjusting for age, sex, and race; model 2 was adjusted for all covariates. In this study, the BMD was quartered in order from small to large (Q1, Q2, Q3, Q4), and the risk of CVD was calculated, respectively. For lumbar BMD, a significant positive association with CVD was found only in the crude model (OR = 3.93, 95% CI 2.12–7.29), and no association was found in either model 1 or model 2 after adjustment. For femur BMD, there was no significant relationship between BMD and CVD risk in the crude model and model 1 ($p = 0.5470$, 0.0585, respectively). After adjusting for age, sex, race, and other relevant covariates (model 2), there was a significant negative association (OR = 0.18, 95% CI 0.06–0.50, $p = 0.0010$). That is, one unit increase in femur BMD was associated with an 82% reduction in CVD risk after adjusting for all relevant covariates. In addition, when BMD was transformed from a continuous variable into a categorical variable (Q1, Q2, Q3, Q4), the trend test was still significant (p for trend = 0.0402). At the same time, with the lowest femur BMD (Q1) as the reference group, the risk of CVD was decreased by 2, 21, and 34% in the Q2, Q3, and Q4 groups, respectively.

The study population was further divided into normal bone mass, osteopenia, and osteoporosis by comparing with the peak BMD of healthy adults of the same sex, to analyze the relationship between osteoporosis and the risk of CVD (15) (Table 3). The study found that osteoporosis was significantly associated with an increased risk of CVD in model 1 and mode 2 (OR = 2.06, 3.05, 95% CI 1.20–3.52, 1.68–5.52, respectively). After adjusting for all potential covariates (model 2), osteoporosis was associated with a 2.05-fold increased risk of CVD compared with the normal group. However, no significant association was found between osteopenia and the risk of CVD.

To further understand the true relationship between BMD and the risk of CVD, the present study also tried to assess the association using a generalized additive model and smooth curve fitting. Figure 2 showed the correlation trend between lumbar BMD and femur BMD and CVD risk, respectively. As can be seen, femur BMD appeared to be curvedly related to CVD risk, while lumbar BMD was not. This non-linear relationship would be further verified next.

Next, a two-piecewise linear regression model was used to analyze the threshold effect. Since the log-likelihood ratio test was $p < 0.05$, we believed that there was a curved relationship between femur BMD and the risk of CVD, and the inflection point was calculated to

2 http://www.r-project.org
3 http://empowerstats.com

TABLE 1 Baseline characteristics of patients (n=2097).

Characteristic	Total	No CVD	CVD	p-value
N	2097	1750	347	
Age	68.92 ± 6.37	68.51 ± 6.25	70.99 ± 6.56	<0.001
Sex				<0.001
Male	1,088 (51.88%)	845 (48.29%)	243 (70.03%)	
Female	1,009 (48.12%)	905 (51.71%)	104 (29.97%)	
Race				<0.001
Mexican American	326 (15.55%)	287 (16.40%)	39 (11.24%)	
Other Hispanic	206 (9.82%)	178 (10.17%)	28 (8.07%)	
Non-Hispanic White	953 (45.45%)	758 (43.31%)	195 (56.20%)	
Non-Hispanic Black	425 (20.27%)	367 (20.97%)	58 (16.71%)	
Other	187 (8.92%)	160 (9.14%)	27 (7.78%)	
SBP, mmHg	134.51 ± 20.30	134.83 ± 20.21	132.91 ± 20.69	0.108
DBP, mmHg	68.73 ± 14.48	69.24 ± 14.66	66.13 ± 13.25	<0.001
BMI, kg/m^2	27.94 ± 5.18	27.78 ± 5.12	28.76 ± 5.37	0.001
WC, cm	99.25 ± 13.44	98.42 ± 13.21	103.42 ± 13.84	<0.001
Hypertension				0.002
No	672 (32.05%)	585 (33.43%)	87 (25.07%)	
Yes	1,425 (67.95%)	1,165 (66.57%)	260 (74.93%)	
Hyperlipidemia				0.017
No	721 (34.38%)	621 (35.49%)	100 (28.82%)	
Yes	1,376 (65.62%)	1,129 (64.51%)	247 (71.18%)	
DM				<0.001
No	1,550 (73.92%)	1,335 (76.29%)	215 (61.96%)	
Yes	547 (26.08%)	415 (23.71%)	132 (38.04%)	
CKD				<0.001
No	2012 (95.95%)	1,697 (96.97%)	315 (90.78%)	
Yes	85 (4.05%)	53 (3.03%)	32 (9.22%)	
Liver disease				0.389
No	1775 (84.64%)	1,476 (84.34%)	299 (86.17%)	
Yes	322 (15.36%)	274 (15.66%)	48 (13.83%)	
Smoke				0.029
No	1740 (82.98%)	1,466 (83.77%)	274 (78.96%)	
Yes	357 (17.02%)	284 (16.23%)	73 (21.04%)	
ALT, U/L	20.00 (16.00–25.00)	20.00 (16.00–25.00)	20.00 (16.00–25.00)	0.251
Albumin, g/L	41.73 ± 3.02	41.79 ± 2.99	41.39 ± 3.14	0.026
AST, U/L	23.00 (20.00–27.00)	23.00 (20.00–27.00)	23.00 (20.00–27.00)	0.238
BUN, mmol/L	5.67 ± 2.34	5.53 ± 2.13	6.37 ± 3.11	<0.001
Creatinine, umol/L	81.33 (69.84–97.24)	79.56 (68.07–93.70)	90.17 (76.91–108.73)	<0.001
Phosphorus, mmol/L	1.20 ± 0.18	1.20 ± 0.18	1.19 ± 0.17	0.342
Calcium, mmol/L	2.36 ± 0.10	2.37 ± 0.10	2.35 ± 0.09	0.027
HDL-c, mmol/L	1.41 ± 0.43	1.43 ± 0.43	1.31 ± 0.39	<0.001
TC, mmol/L	5.12 ± 1.10	5.21 ± 1.08	4.66 ± 1.12	<0.001
HbA1c, %	6.06 ± 1.15	6.01 ± 1.12	6.29 ± 1.26	<0.001
Vitamin D, nmol/L	66.94 ± 27.28	67.37 ± 27.40	64.77 ± 26.56	0.105
Lumbar BMD, gm/cm^2	0.99 ± 0.18	0.99 ± 0.18	1.03 ± 0.18	<0.001
Femur BMD, gm/cm^2	0.91 ± 0.16	0.91 ± 0.16	0.91 ± 0.16	0.547

Mean ± SD or median (IQR) for continuous variables; p-value was calculated by the linear regression model. % For categorical variables; the p-value was calculated by the chi-square test. SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WC, waist circumference; DM, diabetes mellitus; CKD, chronic kidney disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; HDL-c, high-density lipoprotein cholesterol; TC, total cholesterol; HbA1c, glycated hemoglobin.

TABLE 2 Relationship between BMD and CVD.

	Crude model OR (95% CI)	*p*	Model 1 OR (95% CI)	*p*	Model 2 OR (95% CI)	*p*
Lumbar BMD	3.93 (2.12, 7.29)	<0.0001	1.63 (0.81, 3.31)	0.1730	0.97 (0.44, 2.13)	0.9451
Q1	Ref		Ref		Ref	
Q2	1.28 (0.89, 1.83)	0.1865	1.05 (0.72, 1.53)	0.8007	0.98 (0.66, 1.46)	0.9380
Q3	1.74 (1.23, 2.46)	0.0016	1.34 (0.92, 1.94)	0.1226	1.16 (0.78, 1.72)	0.4746
Q4	2.07 (1.48, 2.91)	<0.0001	1.34 (0.92, 1.95)	0.1331	1.06 (0.70, 1.60)	0.7870
p for trend	<0.0001		0.0697		0.6490	
Femur BMD	1.24 (0.61, 2.51)	0.5470	0.43 (0.18, 1.03)	0.0585	0.18 (0.06, 0.50)	0.0010
Q1	Ref		Ref		Ref	
Q2	1.18 (0.85, 1.65)	0.3206	0.99 (0.69, 1.41)	0.9461	0.98 (0.67, 1.42)	0.8964
Q3	1.20 (0.86, 1.67)	0.2883	0.92 (0.63, 1.33)	0.6402	0.79 (0.53, 1.19)	0.2561
Q4	1.27 (0.91, 1.76)	0.1622	0.83 (0.56, 1.23)	0.3574	0.66 (0.43, 1.04)	0.0712
p for trend	0.1818		0.3061		0.0402	

Crude model adjusted for none.
Model 1 adjusted for age, sex, and race.
Model 2 adjusted for age, sex, race, SBP, DBP, BMI, WC, hypertension, hyperlipidemia, DM, CKD, liver disease, smoke, ALT, Albumin, AST, BUN, Creatinine, Phosphorus, Calcium, HDL-c, TC, HbA1c, Vitamin D.
Lumbar BMD: Q1 ≤ 0.863gm/cm^2; Q2 0.864–0.987gm/cm^2; Q3 0.988–1.103gm/cm^2; Q4 ≥ 1.104gm/cm^2.
Femur BMD: Q1 ≤ 0.791gm/cm^2; Q2 0.792–0.902gm/cm^2; Q3 0.903–1.012gm/cm^2; Q4 ≥ 1.013gm/cm^2.

TABLE 3 Relationship between osteoporosis and CVD.

	CVD	No CVD	Crude model	Model 1	Model 2
			OR (95%CI)	OR (95%CI)	OR (95%CI)
Normal	207	1,074	Ref	Ref	Ref
Osteopenia	117	600	1.01 (0.79, 1.30)	1.06 (0.81, 1.37)	1.22 (0.91, 1.64)
Osteoporosis	23	76	1.57 (0.96, 2.56)	2.06 (1.20, 3.52)	3.05 (1.68, 5.52)
P for trend			0.2433	0.0746	0.0034

Crude model adjusted for none.
Model 1 adjusted for age, sex, and race.
Model 2 adjusted for age, sex, race, SBP, DBP, BMI, WC, hypertension, hyperlipidemia, DM, CKD, liver disease, smoke, ALT, Albumin, AST, BUN, Creatinine, Phosphorus, Calcium, HDL-c, TC, HbA1c, Vitamin D.

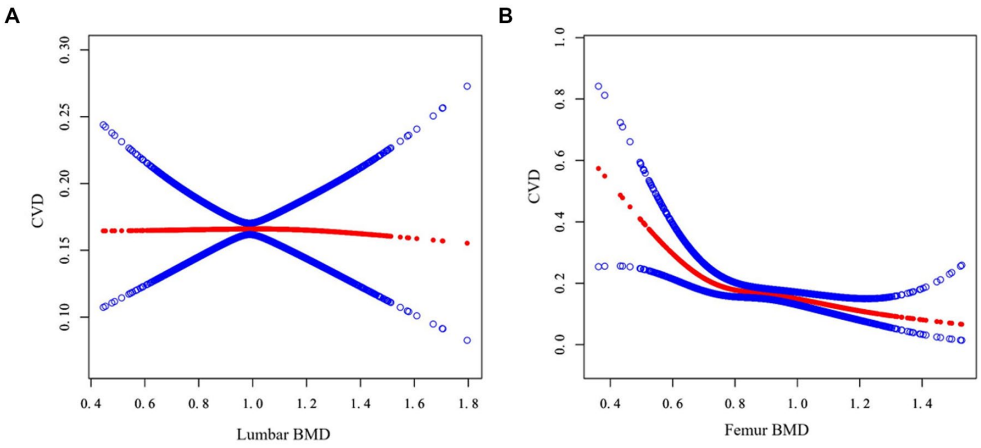

FIGURE 2
Association between BMD and CVD. The abscissa represents the BMD of the lumbar **(A)** and femur **(B)** respectively, and the ordinate represents the risk of developing CVD. The blue area shows the 95% confidence interval.

TABLE 4 Threshold effect analysis of femur BMD and CVD using two-piecewise linear regression.

	Effect size (OR)	95% CI	*p*-value
Model 1			
Fitting by the standard linear model	0.180	(0.065, 0.502)	0.0010
Model 2			
Fitting by the two-piecewise linear model			
Inflection point	0.741		
< 0.741 gm/cm^2	0.003	(0.000, 0.094)	0.0008
> 0.741 gm/cm^2	0.319	(0.105, 0.974)	0.0448
Log-likelihood ratio	0.018		

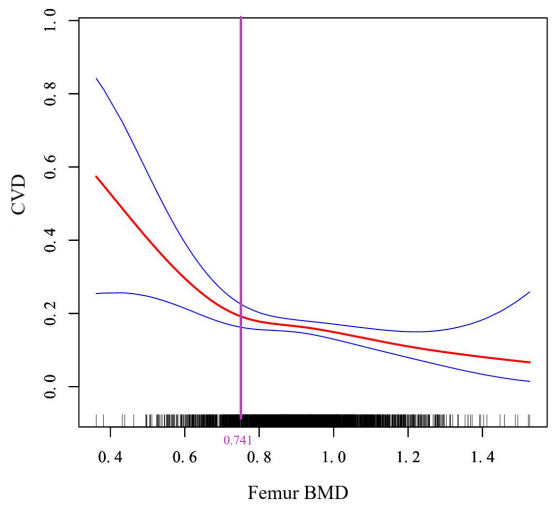

FIGURE 3
Curve association between femur BMD and CVD. The solid red line in the middle represents the trend of CVD risk as the femur BMD increases, and the dashed blue lines on either side represent the 95% confidence interval. The purple vertical line is the calculated inflection point.

be 0.741gm/cm^2 (Table 4). As shown in Figure 3, when BMD <0.741gm/cm^2, the risk of CVD decreased rapidly with the increase of femur BMD. When the femur BMD was >0.741gm/cm^2, the risk of CVD was further reduced, but the rate of decline was slightly slowed (OR = 0.003, 0.319, respectively).

Subsequently, we conducted a subgroup analysis stratified by age, sex, race, body mass index, hypertension, hyperlipidemia, diabetes, chronic kidney disease, liver disease, and smoke, and the results were shown in Table 5. There were no significant differences in the interaction test (*P* for interaction >0.05) except race, indicating that the relationship between femur BMD and CVD was not different between age, gender, and comorbidities.

TABLE 5 Effect size of femur BMD on CVD in subgroups.

	OR	95% CI	*p* for Interaction
Sex			0.2377
Male	0.17	(0.05,0.59)	
Female	0.15	(0.02,0.97)	
Age			0.3111
<70	0.26	(0.06,1.12)	
≥70	0.11	(0.02,0.48)	
Race			0.0480
Mexican American	3.16	(0.20, 50.12)	
Other Hispanic	0.02	(0.00, 3.02)	
Non-Hispanic White	0.17	(0.04, 0.74)	
Non-Hispanic Black	0.03	(0.00, 0.35)	
Other	0.12	(0.00, 15.69)	
BMI			0.5598
<25	0.08	(0.01,0.64)	
≥25, <30	0.21	(0.04,1.10)	
≥30	0.30	(0.05,1.78)	
Hypertension			0.7886
No	0.23	(0.03,2.14)	
Yes	0.17	(0.05,0.57)	
Hyperlipidemia			0.8952
No	0.15	(0.02,1.08)	
Yes	0.18	(0.05,0.61)	
DM			0.7395
No	0.20	(0.05,0.74)	
Yes	0.11	(0.02,0.62)	
CKD			0.8349
No	0.17	(0.06,0.51)	
Yes	0.27	(0.001,86.12)	
Liver disease			0.7677
No	0.16	(0.05,0.48)	
Yes	0.18	(0.01,3.93)	
Smoke			0.3372
No	0.26	(0.08,0.82)	
Yes	0.03	(0.00,0.42)	

Discussion

The relationship between BMD and CVD disease is interesting, and this study does yield some very valuable findings. Firstly, this was a cross-sectional study of 2097 people over 60 years old (mean age 68.92, 51.88% male). After adjusting for all potential confounders, no significant correlation was found between lumbar BMD and CVD, while there was a non-linear relationship between femur BMD and the risk of CVD, with an inflection point of 0.741gm/cm^2. Further, osteoporosis was associated with a significantly increased risk of CVD compared with those with normal bone mass (OR = 3.05, 95% CI 1.68–5.52). In addition, there were no significant differences among different groups of age, sex, body mass index, hypertension, hyperlipidemia, diabetes, chronic kidney disease, liver disease, and smoking. The results of

this study provided powerful evidence support for the risk factors of CVD.

Recent studies have also reported the relationship between BMD and the risk of CVD, which is consistent with the conclusion of this study. Iseri and colleagues found that patients with higher Framingham cardiovascular risk scores tended to have lower head BMD ($p < 0.001$) (16). BMD was also significantly reduced in patients with abnormal myocardial perfusion or impaired left ventricular ejection fraction ($p < 0.05$) (17). Coronary artery calcification is a hallmark pathological change of coronary heart disease. Wiegandt found a negative correlation between BMD and coronary artery calcification (18). Of course, apart from BMD, higher cortical bone status and bone strength were associated with a lower risk of major cardiovascular adverse events after adjusting for confounders (19). In addition, several studies have reported the relationship between BMD and CVD outcomes. For example, a prospective cohort study from the UK Biobank found that osteoporosis was strongly associated with cardiovascular mortality in men (20). A cohort study of chronic heart failure in Japan found that patients with osteoporosis had a significantly increased incidence of adverse events, such as hospitalization or death (HR = 2.40, 95% CI 1.36–4.22) (21). Bisphosphonate is the first-line drug for the treatment of osteoporosis. A recent survey in China found that bisphosphonate significantly reduced the risk of all-cause mortality in patients with acute coronary syndrome or ischemic stroke (22).

CVD is the first major killer threatening human health. The research on its risk factors has been deeply concerned by both researchers and clinicians. According to relevant literature in recent years, BMD itself is also closely related to the main risk factors of CVD. For example, in a cross-sectional study of Japanese women, patients with essential hypertension had significantly lower BMD compared with the control group. Data also showed that BMD was particularly closely related to systolic blood pressure than diastolic blood pressure (23). In a survey of the community population in western China, the relationship between total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol and BMD of postmenopausal women showed a U-shaped curve. That is, on the left side of the inflection point, BMD was negatively correlated with these lipid indexes, while on the right side, they were positively correlated (24). What's more, diabetes status was also related to BMD. Z score of heel BMD in premenopausal women with type 1 diabetes was significantly lower than that in the control group, and serum bone resorption markers, such as tartrate-resistant acid phosphatase-5b, were significantly higher than the control (25). While the respiratory risks of smoking are well known, the effects on BMD are not quite clear. An epidemiological study in South Korea found that tobacco exposure resulted in a significant decrease in BMD ($p < 0.001$), while a healthy lifestyle, such as avoiding sedentary jobs and increasing physical activities, was positively correlated with BMD (26). All of these have demonstrated the internal relationship between BMD and the risk of CVD, which are expected to guide primary prevention and clinical treatment.

In this study, the types of CVD included congestive heart failure and coronary heart disease. On the one hand, they are the most common diseases among the older adults, on the other hand, they are also the main causes of disability and death. Heart failure is a common manifestation of end-stage CVD, including rheumatic heart disease, hypertensive heart disease, myocardial disease, and ischemic heart disease (27). Here are several studies that have looked at BMD and heart failure. Fohtung and colleagues found that lower total hip BMD was associated with a higher risk of heart failure in a population over 65 years of age, and there were differences by gender and ethnicity (28). A large European Norfork epidemiological study found that a 1sd increase in BMD was associated with a 23% reduction in the risk of heart failure (29). A recent meta-analysis also showed that compared with healthy individuals, patients with chronic heart failure had more bone loss and lower total BMD, and further stratified analysis observed similar effects in the femoral neck, arm, leg, and trunk (30). Coronary heart disease (CHD) ranks first among CVD in the older adults and is mediated by atherosclerotic plaque. Angina pectoris and myocardial infarction, as the main clinical types of CHD seriously harm the health of the older adults. To date, few studies have shown a direct link between angina pectoris alone and BMD. However, studies on BMD and myocardial infarction are not scarce. For example, a cross-sectional study from NHANES III in the United States found that low BMD was associated with an increased incidence of myocardial infarction in older adults aged 50–79 years (OR = 1.28, 95% CI 1.01–1.63) (31). However, there are also some contradictory conclusions. For example, Pittman found that increased BMD through the use of anti-resorptive drugs increased the risk of myocardial infarction (HR = 1.38) (32). Though it was not ruled out that the above adverse effects were caused by drug side effects, prospective scientific trials are still needed to further analyze and verify these findings in the future.

It should be noted that no gender difference between BMD and the risk of CVD was observed in the sex-specific stratified analysis (Table 5). However, according to a large number of epidemiological surveys, women were more prone to suffer from osteoporosis than men, especially postmenopausal women (33). Estrogen deficiency was considered to be an important cause, so the guidelines have always recommended estrogen or estrogen receptor modulators for the prevention and treatment of perimenopausal or postmenopausal women (34). The mechanism of estrogen in osteoporosis was quite complex. On the one hand, estrogen stimulated bone formation by acting directly on osteoblasts; On the other hand, estrogen also inhibited osteoclast formation by regulating some cytokines and growth factors. In addition, estrogen also regulated bone metabolism by regulating the expression of various hormones, such as promoting calcitonin secretion and enhancing liver 25-hydroxylase and renal 1α -hydroxylase activities (35). Therefore, in the actual clinical personalized decision-making, the gender difference between patients and menstrual status is also an aspect that doctors must consider and pay attention to.

CVD and osteoporosis are often comorbidities in the older adults. Whether they simply coexist or interact with each other in pathogenesis is still controversial (36). Although the concept of the bone-vascular axis has been proposed for a long time (37, 38) and a growing body of evidence links abnormal BMD or bone metabolism with the risk of CVD, the specific cellular and molecular mechanisms remain unclear. Based on the latest research results from recent years, it may be related to the following aspects: First, there were common risk factors, common genetic and pathological mechanisms, as well as the causal association between osteoporosis

and CVD, so they interacted and influenced each other (39). Second, the presence of vascular calcification might be the most important factor explaining the association between them. Vascular calcification was an active and complex process, especially with age, calcium was gradually lost from the bones and deposited in the cardiovascular system, setting off a host of diseases (40). To be specific, with bone loss, vascular smooth muscle cells transformed into osteoblast phenotype through increasing the level of matrix metalloproteinase-2 and transactivating the RunX promoter (41, 42), which led to vascular calcification and increased hardness, affecting the hemodynamics of the cardiovascular system. What's more, another possible factor was low levels of inflammation, which played a catalytic role in the reduction of BMD (43) and was a crucial role in the pathogenesis of atherosclerotic vascular disease (44). There are several clinical trials targeting inflammation are currently under investigation. Last but not least, people with poor bone health tended to be weaker and less physically active, especially those with combined fractures, and prolonged bedridden conditions significantly increased the risk of CVD (45).

Based on the results of this study, it can be roughly speculated that DXA examination or targeted prevention strategies, such as increased sun exposure, appropriate physical exercise, and calcium or vitamin D supplement, can be considered for patients with CVD. Meanwhile, for patients with osteoporosis or those at high risk of fracture, active anti-osteoporosis drug therapy can increase BMD and improve bone quality and reduce cardiovascular complications to a certain extent. Of course, large randomized controlled trials are needed before BMD measures are widely used to guide the treatment of patients with CVD.

Admittedly, there are some limitations. Firstly, this was a cross-sectional study based on the target population, making it difficult to determine the exact causal relationship between BMD and CVD. Secondly, all the samples used for analysis in this study were from the NHANES database. Although these samples represented the American population well, further research with multi-center data from other countries and regions is still needed. Thirdly, the study excluded patients with malignant tumors and thyroid diseases, which were common causes of secondary osteoporosis, so it was not possible to evaluate the applicability to these populations. Fourthly, the prevalence of CVD in this study was calculated according to the patient's self-reported medical history, which inevitably resulted in recall bias and reporting bias. In addition, due to the limitation of the database, this study only included heart failure and coronary heart disease, so it was not possible to assess the effect of BMD on other CVD. Finally, there were also potential variables not included that may cause bias, such as markers of serum bone turnover, inflammatory parameters, and dietary intake. In recent years, with the standardization of testing procedures, bone turnover markers are increasingly used in the routine management of osteoporosis, especially in pharmacodynamic evaluation (46, 47). International guidelines also recommend its measurement as an alternative to continuous BMD testing in mainstream clinical practice. However, it is still a long way to go to conduct a comprehensive evaluation with multi-center and large sample studies in the future. It should be noted that the normal range of reference values for different populations is also a factor worth considering.

Conclusion

In conclusion, there was a negative non-linear relationship between the level of femur BMD and the prevalence of CVD in people over 60 years of age, with an inflection point of 0.741gm/cm^2. No significant differences were found between age, gender, and comorbidities subgroups. Bone loss can be considered as a new risk factor for CVD, and future studies need to make a comprehensive assessment combining dietary and serum indicators. Therefore, efforts to prevent osteoporosis are of great importance, as this may indirectly reduce the prevalence of CVD, the world's biggest killer of humans.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Protocol #2018-01 NCHS Ethics Review Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

YY: data curation, formal analysis, visualization, and writing—original draft. YH: conceptualization, project administration, supervision, validation, writing—review and editing, and supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Sarcopenia, frailty, and elective surgery outcomes in the elderly: an observational study with 125 patients (the SAFESOE study)

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Background: Sarcopenia is a syndrome characterized by loss of muscle mass, strength and function. Frailty, a state of vulnerability with diminished reserves. The measurement of perioperative risk does not include the assessment of these variables, as little is known about how these conditions impact each other.

Methods: Observational study with a cross-sectional and a prospective cohort component. Elderly people over 60 years of age, able to walk and to independently perform activities of daily living were consecutively recruited in the preoperative period of non-emergency surgical procedures. Frailty was measured by the modified frailty index (mFI-11). Sarcopenia was measured by: (1) thickness and echogenicity on ultrasound; (2) handgrip strength on dynamometry and (3) gait speed. Data obtained from eight muscle groups were submitted to Principal Component Analysis. Postoperative complications were measured using the Clavien-Dindo scale. Follow-up was performed for 1 year to record readmissions and deaths.

Results: Between February and May 2019, 125 elderly people were recruited, median age of 71 years (IQR 65–77), 12% of whom were frail. Frailty was associated with older age, use of multiple medicines, presence of multimorbidity and greater surgical risk according to the American Society of Anesthesiologists (ASA) scale, in addition to lower gait speeds and lower handgrip strength. Frailty was also independently associated with smaller measurements of muscle thickness but not with echogenicity, and with longer hospital and Intensive care unit (ICU) stays. Prevalence of sarcopenia was 14% when considering at least two criteria: low walking speed and low handgrip strength. For muscle thickness, lower values were associated with female gender, older age, frailty, lower gait speeds and lower muscle strength, higher proportion of postoperative complications and higher occurrence of death. For echogenicity, higher values were related to the same factors as those of lower muscle thickness, except for postoperative complications. Lower gait speeds and lower handgrip strength were both associated with higher proportions of postoperative complications, and longer hospital stays. A higher mortality rate was observed in those with lower gait speeds.

Conclusion: Sarcopenia was associated with frailty in all its domains. Unfavorable surgical outcomes were also associated with these two conditions.

KEYWORDS

sarcopenia, frailty, ultrasonography, aged, postoperative complications

1. Introduction

Sarcopenia is a progressive and generalized muscle disease related to aging, characterized by loss of muscle mass, quality, and strength, with an impact on function, resulting in some degree of physical disability, loss of quality of life and increased mortality (1, 2). The prevalence and impact of sarcopenia vary substantially, as they depend on the definition applied, staging, and choice of instruments used for measurement, as well as the age range of the considered population (3). Sarcopenia is believed to be the main risk factor for aging-related functional decline (4, 5). Sarcopenia in the elderly and in patients with several comorbidities is related to prolonged hospitalizations, with higher rates of complications, especially infections, and higher overall mortality (6, 7). Impaired muscle function is an independent predictor of hospitalization, disability, and death (8).

Frailty is a state of vulnerability manifested by diminished physiological reserves that affect the ability to maintain homeostasis in the event of exposure to stressors, resulting in increased risk for adverse health outcomes (9–15). For a fragile patient, stressors can result in serious consequences, such as increased dependence on caregivers and a greater predisposition to falls and delirium. Frailty prevalence rates also vary widely, and this discrepancy can be explained by the lack of consensus on the definition applied, different instruments used for assessment, demographic characteristics, and particularities of the studied population (16, 17).

Elderly patients are at increased risk of postoperative complications (18), which require rapid recognition and treatment, particularly in this population, otherwise they can frequently lead to a cascade of events that may result in loss of independence and worsening of quality of life, higher treatment-related costs, some degree of disability, and greater mortality (19). Studies suggest that frailty is a condition that affects a large proportion of elderly patients who will undergo surgery (20–22).

In this scenario, the present study aimed to evaluate the association between sarcopenia, in its three domains, with frailty, in a population of elderly people admitted to hospital to undergo non-emergency surgical procedures. The association of sarcopenia with frailty is still unclear in the literature. There are studies that show the influence of sarcopenia on frailty overtime (23). Moreover, as a secondary objective, to evaluate the correlation of sarcopenia and frailty with postsurgical outcomes, as sarcopenia and frailty seem to have significant adverse impacts on the occurrence of postoperative outcomes (24).

2. Methods

An observational study was conducted in the preoperative units of the Hospital Sírio-Libanês, in São Paulo, Brazil–a tertiary, philanthropic institution with a 500-bed capacity. The participants were elderly people over 60 years of age, able to walk, with or without walking aids, admitted to hospital for non-emergency surgical procedures, who signed the Free and Informed Consent Term. Those with neuromuscular diseases, paresis, paralysis or limb amputation, dementia, and that were dependent for daily life activities, or lived with homecare, or were institutionalized were excluded. Patients were recruited consecutively, without prior selection. The initial interviews and measurements took place on the eve of the procedure and lasted an average of 20 min.

In the preoperative period, data were collected to characterize the population (Appendix 1 in Supplementary material). Frailty was characterized by the Modified Frailty Index 11 (which considers the presence of comorbidities and the functional status during the 30 days before surgery). Each one of the present comorbidities add one point, frailty adds on 3 or more points, and pre-frailty puts on 1 or 2 points (16, 17). The assessment of muscle mass was characterized using ultrasound measurement of the cross-sectional area of eight muscle groups: biceps brachii and brachii; rectus femoris; vastus intermedius; rectus abdominis; external and internal obliques; transversus abdominis; and medial gastrocnemius (Appendix 2 in Supplementary material). The percentage of fat was assessed by bioimpedance test (25). Muscle strength was measured with the use of a handgrip dynamometer (8, 9). Muscle function assessed by the 15-meter walk test (16, 17, 25, 26).

In the postoperative period, data related to surgical complications were collected daily, using the Clavien-Dindo Classification (Appendix 3 in Supplementary material), from the day after surgery until discharge (27). Hospital mortality and total length of stay were also computed. Mortality after discharge and hospital readmissions were accessed through telephone contact with the family or guardians, on the first, third and twelfth month after discharge.

Regarding the statistical analysis, categorical data were summarized by their absolute and relative frequencies and compared with the use of the Pearson's Chi-square or Fisher's Exact Test. Continuous data were summarized by their mean and standard deviations or by median and inter-quartile percentiles. When the data had a normal distribution, the Student's t-test was used to compare two independent groups and the Analysis of Variance (ANOVA) and the F-test were used to compare three or more groups. When data were not normally distributed, the Wilcoxon-Mann–Whitney test was used when comparing two independent groups and the Kruskal-Wallis test was used when comparing three or more groups.

For the analysis of data on the thickness and echogenicity of muscle groups, we used the Principal Component Analysis (PCA). PCA was used to explore the correlation structure between the thickness and echogenicity measurements. In addition, it allowed us to reduce the dimension of the problem, by producing two new variables (principal components) able to explain a substantial amount of the original variation, registered by the eight thickness (or echogenicity). Parallel analysis was used to decide the number of principal components to be retained and studied. In the parallel analysis, new datasets are simulated completely at random, and the corresponding components are obtained. Then, the original components are compared with the simulated ones, and only those explaining more than the simulated ones should be considered. The principal components are defined by interpretable linear combinations based on the original variables, where the coefficients of each original variable are related to the correlations between the original variables and the components. Principal component analysis is a usual multivariate statistical method, largely used in medical sciences (28, 29).

The Hypothesis Tests of the association of the scores of the components 1 and 2 were performed separately with the main variables of the study, with the use of the tests for means described

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above. The Spearman's correlation coefficient was calculated for the continuous variable "length of stay." Interactions were also evaluated. Conclusions were based on a 5% significance level. All analysis were performed using the R statistical software, version 4.0.292. The R package psych was used to perform the principal component and parallel analysis.

3. Results

Between February and May 2019, 125 elderly people were recruited during the preoperative period of non-emergency surgeries. The studied group of patients had a slight male predominance (52.8%) and an average age of 71 years (IQR 65–77). The patients used a median of 4 different medications (IQR 2–6), had an average of 3 comorbidities (IQR 2–4), and an ASA score distributed as follows: 12 (9.6%) at level 1; 85 (68%) at level 2; and 28 (22.4%) at level 3. Regarding frailty, 77 (61.6%) were not frail, 33 (26.4%) were pre-frail, and 15 (12%) were frail. It was observed that older people, those who took more medications, had more comorbidities, and had higher ASA scores were more often considered frail (value of ps ≤0.001).

Of the 125 patients, 34 (28.3%) had low gait speed (the cut-off points for gait speed was ≤0.8 m/s). There were 20 (16%) patients with handgrip strength below the expected limit (the cut-off points for dynamometry were ≤ 27 kgf for men and ≤ 16 kgf for women). There were 38 (30.4%) patients with gait speed below 0.8 m/s or handgrip strength below the expected limit. Sarcopenia, measured by the 15-meter gait speed test and by the handgrip strength dynamometry, was positively correlated with frailty (Table 1).

The other parameters used to characterize sarcopenia, which were muscle thickness and echogenicity obtained by ultrasound, are presented in Table 2, separated into the degrees of frailty. Lower values of muscle thickness were associated with frailty, except for the biceps and brachii, medial gastrocnemius, and rectus abdominis. Nevertheless, for echogenicity, there was no relationship between this measurement and frailty.

In Table 3, we describe the relationship between frailty and unfavorable outcomes. There was a significant correlation between frailty and length of stay, and, also, postoperative complications. The most frequent types of complications, considering the Clavien-Dindo Classification, were grades I (20.8%) and II (11.2%). There was no correlation between frailty and readmissions or death, considering the one-year follow-up period.

Table 4 shows that lower gait speeds were associated with higher proportions of postoperative complications, longer length of stay, and higher occurrence of death. Similarly, Table 5 shows that lower handgrip strengths were associated with higher proportions of postoperative complications and longer length of stay, but there were no associations with readmission and death rates.

For the summary of the analysis of the eight muscle groups of the entire studied population, we used the statistical method Principal Component Analysis. Two principal components (or dimensions) were identified. The first principal component (PC1) assigned positive loadings for all thickness and echogenicity variables, i.e., all muscles were positively correlated with each other, that is, in an individual, if a muscle tended to be thinner or more echogenic, such characteristics tended to be replicated in other muscle groups. In other words, there is a congruence of measurements across the eight muscle groups. Vectors in Figure 1 (thickness and echogenicity) are all pointing toward the same direction in relation to the horizontal axis. The second principal component (PC2), on the other hand, assigned loadings with different sets of variables, and it can be interpreted as a contrast or divergence between the muscular behavior for appendicular and abdominal muscles. Component 2 is represented by the fact that the arrows for the appendicular muscles and those of the abdominal muscles are pointing toward opposite direction in relation to the vertical axis. The two principal components jointly explain 58.728 and 65.084% of the original variance for the thickness and echogenicity variables, respectively.

The remaining results show the association of the principal components with the other study variables. For muscle thickness, higher values of component 1 were associated with male gender, younger age, lower degrees of frailty, higher gait speeds and normal handgrip strength measurements by dynamometry, absence of postoperative complications of grade II or higher on the Clavien-Dindo Classification, and not dying. There was no association of the component 1 with readmission for up to 1 year. There was a correlation of the component 2 with gender, that is, the thickness divergence between the appendicular and abdominal muscles occurred differently between the sexes: for men, the thickness of the appendicular muscles is greater than those presented by women. There were no other associations of the component 2 with other variables (Table 6).

TABLE 1 Distribution of measurements of gait speed and strength, by degrees of frailty.

Categories*	Normal (*N* = 77)	Pre-frail (*N* = 33)	Frail (*N* = 15)	Total (*N* = 125)	*p*-value**
Gait speed (m/s)					
	1.1 (0.9–1.2)	0.9 (0.6–1.1)	0.6 (0,3–0.8)	1.0 (0.8–1.2)	<0.001
Gait speed – cut-off point of 0.8 m/s					
<=0.8 m/s	13 (17.6)	10 (32.3)	11 (73.3)	34 (28.3)	<0.001
>0.8 m/s	61 (82.4)	21 (67.7)	4 (26.7)	86 (71.7)	
*Dynamometer – handgrip strength – different cut-off points by sex****					
Normal	72 (93.5)	26 (78.8)	7 (46.7)	105 (84)	<0.001
Below the cut-offs	5 (6.5)	7 (21.2)	8 (53.3)	20 (16)	

*Data are presented as median (IQR) for continuous measures and n (%) for categorical measures.
**Kruskal–Wallis test for continuous measurements and Fisher's exact or chi-square test for categorical measurements.
***Cut-off points for dynamometry: < or > =27 kgf for men and < or > =16 kgf for women.

TABLE 2 Distribution of measurements of each muscle thickness and echogenicity, by degrees of frailty.

Muscles*	Normal (*N* = 77)	Pre-frail (*N* = 33)	Frail (*N* = 15)	Total (*N* = 125)	*p*-value**
*Thickness (cm)**					
Biceps brachii and Brachialis	4.0 (3.7–4.5)	4.1 (3.7–4.6)	3.5 (3.2–4.4)	4.0 (3.6–4.5)	0.15
Rectus femoris	1.2 (1.0–1.5)	1.2 (1.0–1.5)	0.9 (0.8–1.3)	1.2 (1.0–1.5)	0.020
Vastus intermediate	1.1 (0.9–1.4)	1.2 (0.9–1.5)	0.8 (0.7–1.1)	1.1 (0.9–1.4)	0.002
Quadriceps femoris	2.3 (2.0–2.8)	2.5 (2.0–2.9)	1.7 (1.5–2.2)	2.3 (1.9–2.8)	0.005
Rectus abdominis	0.8 (0.7–1.0)	0.9 (0.7–1.0)	0.9 (0.7–0.9)	0.8 (0.7–1.0)	0.81
External oblique	0.5 (0.4–0.6)	0.5 (0.4–0.6)	0.4 (0.3–0.5)	0.5 (0.4–0.6)	0.002
Internal oblique	0.5 (0.4–0.7)	0.5 (0.4–0.8)	0.4 (0.3–0.5)	0.5 (0.4–0.7)	0.004
Transversus abdominis	0.4 (0.3–0.4)	0.4 (0.2–0.4)	0.2 (0.2–0.3)	0.3 (0.3–0.4)	<0.001
Medial gastrocnemius	1.5 (1.3–1.7)	1.6 (1.3–1.7)	1.3 (1.1–1.5)	1.5 (1.3–1.7)	0.060
*Echogenicity (grayscale)**					
Biceps brachii and Brachialis	60.5 (51.8–68.1)	62.9 (53.5–68.5)	64.2 (58.6–72.7)	61.8 (52.8–68.5)	0.35
Rectus femoris	52.9 (44.1–62.5)	54.4 (45.1–63.5)	55.9 (50.4–73.1)	53.5 (45.1–63.3)	0.57
Vastus intermediate	49.9 (41.0–61.8)	47.3 (36.7–62.8)	64.7 (50.2–79.1)	50.8 (38.7–63.9)	0.092
Quadriceps femoris	57.7 (51.6–67.3)	59.4 (53.3–68.4)	65.1 (56.6–76.3)	59.4 (52.1–68.2)	0.19
Rectus abdominis	61.9 (51.1–73.9)	64.5 (55.8–76.7)	61.5 (47.0–71.9)	63.3 (52.5–74.3)	0.48
External oblique	70.3 (59.2–80.9)	71.7 (62.9–79.2)	69.4 (66.8–76.2)	69.9 (62.5–80.0)	0.92
Internal oblique	51.0 (43.0–64.1)	50.9 (47.9–59.3)	57.2 (46.8–64.7)	52.0 (44.9–64.1)	0.57
Transversus abdominis	46.9 (38.6–53.0)	47.4 (35.5–57.1)	56.1 (42.7–60.5)	47.8 (38.7–56.1)	0.081
Medial gastrocnemius	46.3 (40.4–52.4)	48.4 (35.4–54.8)	52.5 (44.7–66.7)	47.0 (40.4–55.3)	0.17

*Data are presented as median (IQR).
**Kruskal–Wallis tests.

TABLE 3 Distribution of postoperative complications, length of stay, readmissions, and death up to 1 year of follow-up, separated by degrees of frailty.

Categories*	Normal (*N* = 77)	Pre-frail (*N* = 33)	Frail (*N* = 15)	Total (*N* = 125)	*p*-value**
Postoperative complications – Clavien-Dindo scale					
No complications	51 (66.2%)	19 (57.7%)	5 (33.3%)	75 (60.0%)	0.049
I	13 (16.9%)	10 (30.3%)	3 (20.0%)	26 (20.8%)	
II	7 (9.1%)	1 (3.0%)	6 (40.0%)	14 (11.2%)	
IIIa	1 (1.3%)	1 (3.0%)	1 (6.7%)	3 (2.4%)	
IIIb	1 (1.3%)	0 (0.0%)	0 (0.0%)	1 (0.8%)	
Iva	2 (2.6%)	1 (3.0%)	0 (0.0%)	3 (2.4%)	
IVb	1 (1.3%)	0 (0.0%)	0 (0.0%)	1 (0.8%)	
V	1 (1.3%)	1 (3.0%)	0 (0.0%)	2 (1.6%)	
Length of stay (days)					
	2.0 (1.0–3.0)	2.0 (1.0–4.0)	4.0 (2.0–15.0)	2.0 (1.0–4.0)	0.037
Readmission within 1 year of hospital discharge					
No	50 (64.9%)	25 (75.8%)	10 (66.7%)	85 (68.0%)	0.392***
Yes	24 (31.2%)	7 (21.2%)	3 (20.0%)	34 (27.2%)	
Not informed	3 (3.9%)	1 (3.0%)	2 (13.3%)	6 (4.8%)	
Death					
No	74 (96.1%)	32 (97.0%)	14 (93.3%)	120 (96.0%)	0.645
Yes	3 (3.9%)	1 (3.0%)	1 (6.7%)	5 (4.0%)	

*Data are presented as median (IIR) for continuous measures and n (%) for categorical measures.
**Kruskal–Wallis test for continuous measurements and Fisher's Exact or Chi-square tests for categorical measurements.
***Excluding the "not informed" category, the p value was equal to 0.516.

TABLE 4 Distribution of the studied outcomes: length of stay (days), readmission, and death due to any cause in up to 1 year of follow-up, separated into two categories of gait speed.

Categories*	Gait speed			*p*-value**
	>0.8 m/s (*N* = 86)	<=0.8 m/s (*N* = 34)	Total (*N* = 120)	
Postoperative complications – binary variable				
No	59 (68.6%)	16 (47.1%)	75 (62.5%)	0.037
Yes	27 (31.4%)	18 (52.9%)	45 (37.5%)	
Length of stay (days)				
	2.0 (1.0–3.0)	4.0 (2.0–10.0)	2.0 (1.0–4.0)	<0.001
Readmission within 1 year of hospital discharge				
No	61 (70.9%)	21 (61.8%)	82 (68.3%)	0.605***
Yes	22 (25.6%)	11 (32.3%)	33 (27.5%)	
Not informed	3 (3.5%)	2 (5.9%)	5 (4.2%)	
Death				
No	85 (98.8%)	30 (88.2%)	115 (95.8%)	0.022
Yes	1 (1.2%)	4 (11.8%)	5 (4.2%)	

*Data are presented as median (IIR) for continuous measures and *n* (%) for categorical measures.
**Kruskal-Wallis test for continuous measurements and Fisher's Exact or Chi-square tests for categorical measurements.
***Excluding the "not informed" category, the *p* value was equal to 0.705.

TABLE 5 Distribution of outcomes studied: length of stay (days), readmissions, and death due to any cause within up to 1 year of follow-up, separated into two categories of handgrip strength.

Categories*	Handgrip strength (dynamometer)			*p*-value**
	Normal (*n* = 105)	Below the cut-offs (*N* = 25)	Total (*N* = 120)	
Postoperative complications – binary variable				
No	68 (64.8%)	7 (35.0%)	75 (60.0%)	0.023
Yes	37 (35.2%)	13 (65.0%)	50 (40.0%)	
Length of stay (days)				
	0.0 (0.0–0.0)	0.5 (0.0–2.0)	0.0 (0.0–0.0)	<0.001
Readmission within 1 year of hospital discharge				
No	71 (67.6%)	14 (70.0%)	85 (68.0%)	0.331***
Yes	30 (28.6%)	4 (20.0%)	34 (27.2%)	
Not informed	4 (3.8%)	2 (10.0%)	6 (4.8%)	
Death				
No	102 (97.1%)	18 (90.0%)	120 (96.0%)	0.181
Yes	3 (2.9%)	2 (10.0%)	5 (4.0%)	

*Data are presented as median (IIR) for continuous measures and *n* (%) for categorical measures.
**Kruskal-Wallis test for continuous measurements and Fisher's Exact or Chi-square tests for categorical measurements.
***Excluding the "not informed" category, the *p*-value was equal to 0.811.

For echogenicity, higher values of component 1 were associated with female gender, older age, higher degrees of frailty (although only frailty categorized as a binary variable had a value of p <5%), lower gait speeds, lower handgrip strength measurements by dynamometry, and death (Table 6). There was an association of the component 2 with female gender, lower gait speeds and lower handgrip strength measurements by dynamometry, in addition to death - meaning that the echogenicity of the appendicular muscles, relative to those of the axial muscles, was higher for women, for people with low walking speed gait and low handgrip strength by dynamometry (Table 6).

4. Discussion

This study aimed to establish the association between frailty and sarcopenia in a series of 125 elderly subjects in the preoperative period of non-emergency surgery. As a secondary objective, we also sought to correlate sarcopenia and frailty with unfavorable outcomes, such as: longer hospital stays, readmissions, death, and postoperative complications.

Considering frailty, the prevalence found in our study was 12%, similar to other studies, which indicate the general prevalence of

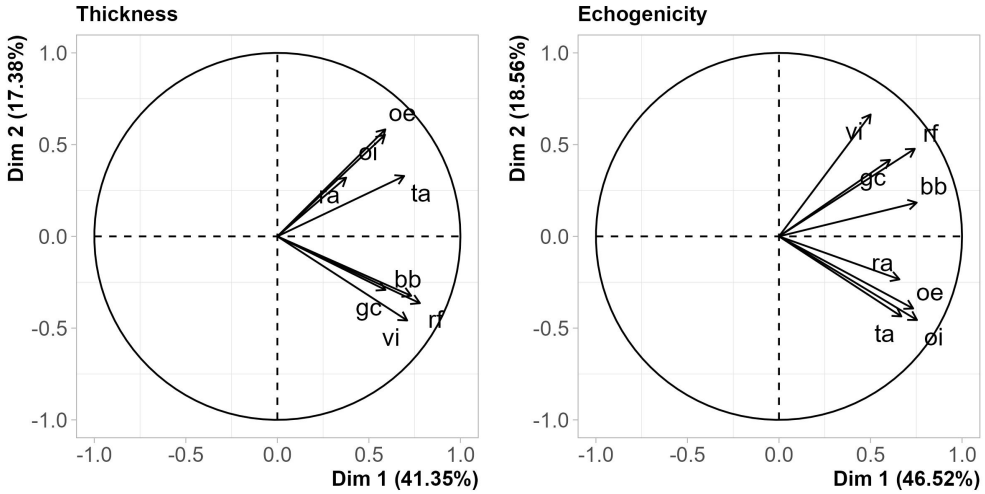

FIGURE 1
Principal component analysis of thickness and echogenicity measurements. The horizontal axis stands for the first principal component, the vertical, the second, whereas the arrows represents the original variables. Arrows with similar orientations show positively correlated variables.

frailty from 10.7 to 13.6%. Interestingly enough, the prevalence of frailty in the elderly population varies from 4.0 to 59.1%, depending on the definition used and the population studied (16). The scale chosen to assess frailty in our study was the Modified Frailty Index 11 (mF11), which is already well established in the literature, and represents an accurate predictor of mortality and postoperative complications in surgery (30).

In our study, frailty was related to older age, but not to gender. This finding is coincident with the literature. Criteria for frailty were found in 16.7% of males and 6.8% of females (Appendix 1 in Supplementary material), differing from authors such as Collard and Fried, who found that the prevalence of frailty increases with age but is higher in women (16, 17, 31).

The prevalence of sarcopenia was 30.4% (Table 1), with no significant gender differences, when considering at least one criterion: gait speed below 0.8 m/s or handgrip strength below the expected limit for sex group. When considering at least two criteria, as recommended by the European consensus, the prevalence of sarcopenia in our study approaches the value of 14%, similar to that observed in the literature (32). The prevalence of sarcopenia varies enormously depending on the definition and instrument used to define it and the population studied. In studies carried out in the general population, the prevalence of sarcopenia in adults aged 60 to 70 years varies between 5 and 13%, increasing to 11 to 50% in those aged 80 years and over (33). In a meta-analysis that pooled studies with the definition of sarcopenia based on the European Working Group on Sarcopenia in Older People (EWGSOP) 2019 consensus, in healthy patients, aged 60 years and over, the overall estimates were 3.96% (32). In our study, the prevalence of sarcopenia, as defined by the European consensus, was slightly higher than that of frailty. It is important to keep in mind that frailty constitutes a broader functional spectrum compared to sarcopenia (34).

Frailty was correlated with sarcopenia, both in terms of loss of muscle strength and low gait speed, both conditions predefined by the EWGSOP consensus (32). Regarding the muscle mass criterion, frailty was correlated with smaller thicknesses of several muscles when evaluated separately, and there was also an association of frailty (binary variable) with component 1. It was previously seen that an increase in echogenicity of the quadriceps femoris is positively associated with age and negatively associated with muscle thickness and isometric knee extensor strength (35). In a big cohort of healthy men, the increase of echogenicity of the anterior thigh muscle compartment was related to diminished muscle strength (36). Nevertheless, in our study, no correlation was observed between echogenicity, and frailty. To justify our finding, it is important to point out that the most expressive gain in echogenicity already occurs in middle-aged individuals and may precede the loss of muscle mass (37).

Regarding the surgical outcomes associated with frailty, non-frail patients had fewer surgical complications, as assessed by the Clavien-Dindo Classification scale. There was a positive relationship between longer hospital stay and frailty. Readmissions within one year of follow-up were not correlated with frailty. No statistical correlation between death and frailty was observed. Studies indicate that the aging process varies between individuals depending on their overall functional reserves. This is reflected, for example, in a higher risk of poorer outcomes, given a surgical intervention, including greater chances of mortality (38).

Makary et al. studied patients undergoing elective surgery and demonstrated an increased risk of bad outcomes related to an increasing degree of frailty (39). McAdams-DeMarco et al. also demonstrated that frail patients undergoing kidney transplantation were significantly more likely to be readmitted earlier to the hospital, regardless of age (40). Frailty was directly related to an increase in the number of complications related to colorectal, cardiac and emergency surgery (41, 42).

In our study, considering the same outcomes, now faced with sarcopenia, lower gait speeds were associated with higher proportions of postoperative complications (Clavien-Dindo Classification equal to or above II), longer total hospital stay, and higher death rates. In the sarcopenia (loss of strength criterion), lower handgrip strengths were associated with higher incidence of post-surgical complications: Clavien-Dindo Classification equal to or above II and longer total hospital stay. No association with readmission or death was observed.

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TABLE 6 Association between the principal components that summarize the thickness and echogenicity measurements and other categorical variables.

Variable	Category	*N* (%)	Comp. 1*	*p*-value (1)**	Comp. 2*	*p*-value (2)**
Thickness						
Sex	Male	66 (52.8)	0.648 (1.777)	<0.001	−0.444 (1.199)	<0.001
	Female	59 (47.2)	−0.725 (1.605)		0.497 (0.953)	
Age	(60–69]	58 (46.4)	0.697 (1.713)	<0.001	0.102 (1.217)	0.666
	(70–79]	46 (36.8)	−0.250 (1.701)		−0.076 (1.189)	
	(80+]	21 (16.8)	−1.377 (1.502)		−0.117 (1.109)	
Frailty binary	Normal	110 (88.0)	0.240 (1.704)	0.001	0.035 (1.195)	0.354
	Frail	15 (12.0)	−1.761 (1.774)		−0.255 (1.098)	
Frailty categories	Normal	77 (61.6)	0.190 (1.587)	<0.001	0.069 (1.217)	0.608
	Pre-frail	33 (26.4)	0.358 (1.973)		−0.045 (1.159)	
	Frail	15 (12.0)	−1.761 (1.774)		−0.255 (1.098)	
Gait speed	>0.8 m/s	86 (71.7)	0.541 (1.649)	<0.001	−0.032 (1.206)	0.713
	<=0.8 m/s	34 (28.3)	−1.429 (1.572)		0.055 (1.149)	
Dynamometry	Normal	105 (84.0)	0.385 (1.615)	<0.001	0.008 (1.226)	0.848
	Weak	20 (16.0)	−2.023 (1.536)		−0.040 (0.957)	
Postoperative complications	No	75 (60.0)	0.187 (1.702)	0.176	−0.061 (1.270)	0.464
	Yes	50 (40.0)	−0.280 (1.982)		0.092 (1.047)	
Readmission	No	85 (71.4)	−0.062 (1.784)	0.560	0.084 (1.139)	0.483
	Yes	34 (28.6)	0.157 (1.858)		−0.089 (1.233)	
Death	No	120 (96.0)	0.087 (1.796)	0.019	0.012 (1.204)	0.254
	Yes	5 (4.0)	−2.080 (1.328)		−0.283 (0.465)	
Echogenicity						
Sex	Male	66 (52.8)	−0.669 (1.991)	<0.001	−0.288 (1.153)	0.005
	Female	59 (47.2)	0.748 (1.581)		0.322 (1.229)	
Age	(60–69]	58 (46.4)	−0.481 (1.921)	0.032	−0.142 (1.169)	0.287
	(70–79]	46 (36.8)	0.469 (1.863)		0.020 (1.186)	
	(80+]	21 (16.8)	0.302 (1.914)		0.350 (1.427)	
Frailty binary	Normal	110 (88.0)	−0.102 (1.994)	0.035	−0.038 (1.167)	0.474
	Frail	15 (12.0)	0.745 (1.266)		0.276 (1.598)	
Frailty categories	Normal	77 (61.6)	−0.162 (1.964)	0.252	−0.018 (1.145)	0.631
	Pre-frail	33 (26.4)	0.040 (2.085)		−0.084 (1.236)	
	Frail	15 (12.0)	0.745 (1.266)		0.276 (1.598)	
Gait speed	>0.8 m/s	86 (71.7)	−0.489 (1.984)	<0.001	−0.166 (1.170)	0.018
	<=0.8 m/s	34 (28.3)	1.120 (1.279)		0.445 (1.257)	
Dynamometry	Normal	105 (84.0)	−0.245 (1.912)	<0.001	−0.123 (1.162)	0.026
	Weak	20 (16.0)	1.286 (1.551)		0.646 (1.360)	
Postoperative complications	No	75 (60.0)	−0.091 (1.998)	0.516	−0.134 (1.219)	0.133
	Yes	50 (40.0)	0.137 (1.854)		0.202 (1.215)	
Readmission	No	85 (71.4)	0.071 (1.977)	0.384	0.027 (1.192)	0.600
	Yes	34 (28.6)	−0.271 (1.895)		−0.098 (1.149)	
Death	No	120 (96.0)	−0.034 (1.968)	0.010	−0.035 (1.230)	0.048
	Yes	5 (4.0)	0.827 (0.477)		0.832 (0.703)	

*Mean (standard deviation) for the scores of the respective principal components.
**Mean comparison tests (*t* test for two groups and *F* test for three groups).

The mortality rate up to 1 year after surgery was 4% in our study group and it was especially related to sarcopenia (80% of these patients had severe sarcopenia). Of the 34 patients who underwent readmissions, 43.2% had at least 1 criterion to define sarcopenia or frailty.

5. Conclusion

In our study, sarcopenia, in all its domains, was associated with frailty. Unfavorable surgical outcomes were also associated with these two conditions.

Our findings also suggest that screening for sarcopenia and frailty in elderly patients who will undergo elective surgery is relevant, easy-to-perform and helps to access perioperative risk in this population.

We recognize, as limitations of this study, that this is a small sample in which the recruitment occurred in a single private hospital, that may cause restrictions to the generalization of our findings.

The present study is the first, to our knowledge, to assess sarcopenia in all its domains, with frailty, in a presurgical elderly population, with a 1 year follow up. The use of well-defined cutoff parameters, by the latest consensus of the EWGSOP 2019 contributes to create a more universal language for future studies in this area.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Hospital Sirio Libanes. The patients/participants provided their written informed consent to participate in this study.

Author contributions

IS: conception and design of the work and acquisition of data for the work. CT and JS: substantial contributions to the analysis and interpretation of data for the work. CB: revising the article–intellectual content. AA: substantial contributions on acquisition of data for the work. RS: substantial contributions to the conception or design of the work. AB: substantial contributions to the conception or design of the work and accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1185016/full#supplementary-material

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Impact of exercise and leucine-enriched protein supplementation on physical function, body composition, and inflammation in pre-frail older adults: a quasi-experimental study

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Background: Exercise and a protein-enriched diet are essential for muscle protein synthesis, cellular growth, mitochondrial function, and immune function. The U.S. Food and Nutrition Board's current guideline on recommended dietary allowance for protein in older adults is 0.8 g/kg per day, which may not be sufficient in vulnerable pre-frail older adults.

Aims: This study aimed to evaluate the impact of leucine-enriched protein supplementation with or without exercise over 3 months in pre-frail older adults who consumed ≤1 g/kg/day of protein on improving (i) physical function, (ii) body composition measures, and (iii) inflammatory biomarkers such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α).

Methods: A non-randomized cluster quasi-experimental study guided by the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist of 178 pre-frail older adults [112 control, 44 nutrition (Nu), and 22 in the nutrition with exercise (Nu+Ex) group] comparing the effect of Nu+Ex and Nu on physical function, body composition, and inflammation. At 0, 3, and 6 months, questionnaires on demographics, depression, perceived health, and cognition were administered. Physical function assessment (short physical performance battery [SPPB] test, gait speed, handgrip strength, 5× sit-to-stand [STS]) was conducted, and body composition analysis was performed using a bioelectrical impedance analysis machine. IL-6 and TNF-α were measured at 0 and 3 months.

Results: At 3 months, there were significant improvements in gait speed, 5× STS, SPPB scores, depression, perceived health, fat-free mass, and appendicular skeletal muscle mass indices in the Nu+Ex group. Both Nu+Ex and Nu groups had improvements in body cell mass and reductions in IL-6 and TNF-α. The improvements were not sustained after 6 months.

Conclusion: Our study results need to be validated in future longitudinal randomized studies with a larger sample size focusing on populations at risk.

KEYWORDS

leucine-enriched protein supplement, pre-frail, tumor necrosis factor alpha, interleukin-6, body composition, physical function

1. Introduction

The world's population is aging at an unprecedented rate, especially in the Asia-Pacific region, where countries such as Singapore and South Korea have the fastest aging population (1). Worldwide, the number of older adults 65 years of age and older is expected to double to 1.5 billion by 2050 and may account for 33.3% of the population in Singapore during the same time frame (2, 3). Aging is associated with declines in physical function, anorexia of aging, sarcopenia, and frailty, which are major contributors to healthcare costs (4). Frailty is a state of declining physiological reserve that predisposes older adults to an increased risk of adverse outcomes, while sarcopenia is defined as a loss of muscle function, quality, or mass. It is a component of physical frailty (5, 6). Pre-frail is a transition phase to frailty, where one in five may progress to frailty over 3 years. The progression is associated with double the healthcare cost, and pre-frailty may be reversible before the onset of disability (4, 7). The combination of a sedentary lifestyle, increased prevalence of multimorbidity, and low protein and energy intake secondary to anorexia of aging are well-recognized factors contributing to the loss of muscle mass, frailty, and disability in older adults (8). Nutrition and exercise are well-recognized modifiable factors for a longer health span, especially in sarcopenic, and frail older adults. There are multiple factors associated with low energy and protein intake in older adults, such as poor oral health, multimorbidity with superimposed dietary restriction, limited access to food, financial insecurity, polypharmacy, and dysregulation of gut peptide releases such as ghrelin, leptin, and insulin (9, 10).

Protein is a vital nutrient in the diet of older adults. It is responsible for maintaining good oral health, musculoskeletal function, immune function, wound healing, and improving insulin sensitivity (9). Leucine is a branched-chain amino acid, and leucine-enriched protein supplements, together with resistance exercise, have been shown to potentiate muscle protein synthesis, reduce anabolic resistance, and improve muscle mass and physical function in healthy and sarcopenic older adults (11, 12). Since the COVID-19 pandemic, the role of a protein-enriched diet and exercise has become even more important, specifically to restore muscle mass loss caused by lockdown and movement restrictions (13). The U.S. Food and Nutrition Board's current guideline on the recommended dietary allowance (RDA) for protein in older adults is 0.8 g/kg per day. However, it is well known that older adults require much higher protein than the RDA due to body composition changes, low-grade inflammation, multimorbidity, insulin resistance, and a higher propensity to develop anabolic resistance (14–16).

A high protein diet supplemented by leucine and/or vitamin D has been shown to reduce proinflammatory biomarkers such as chemerin and progranulin in diabetics and attenuate the rise of interleukin-6 (IL-6) in sarcopenic older adults (17, 18). The role of resistance exercise in combination with leucine-enriched protein supplementation in improving muscle mass, physical function, and inflammatory biomarkers such as IL-6 and tumor necrosis factor-alpha (TNF-α) has shown mixed results in frail or pre-frail older adults who are at greatest risk of functional decline (19). Most prior studies have either focused on healthy or sarcopenic older adults, and there are limited studies on lower baseline protein consumption as inclusion criteria. This study aimed to evaluate if a diet enriched with an additional 16 g of protein and 3 g of leucine supplementation with or without exercise over 3 months in pre-frail older adults who consume ≤1 g/kg of protein a day improves (i) physical function, (ii) fat-free mass (FFM) and appendicular skeletal muscle (ASM) mass, and (iii) systemic inflammation as measured by IL-6 and TNF-α.

2. Materials and methods

2.1. Participants and study design

This study was originally designed as a three-cluster randomized control trial but had to be converted to a non-randomized quasi-experimental design due to COVID-19 restrictions and lockdown. The initial sample size calculations of at least 65 participants per group were based on 80% power and two-sided 5% significance on a conservative Interclass Correlation Coefficient (ICC) of 0.02 and a Cohen's effect size of 0.5 in the gait speed and muscle mass differences between the three groups. The important modifications in response to extenuating circumstances were approved by the local ethics board and did not affect the research question or outcomes (20). It impacted mainly the change in the control population, which was part of another study with similar inclusion criteria. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist for observational studies was used to guide the reporting of the findings (21, 22), and the baseline differences were adjusted for in the final analysis.

The inclusion criteria included participants ≥60 years old, able to provide consent and adhere to instructions as deemed suitable by a primary care physician or trained members of the study team, ambulant, and pre-frail. Participation was entirely voluntary. Participants with a pacemaker or a defibrillator, liver or gastrointestinal disease, end-stage lung disease, cardiac disease, cancer undergoing active treatment, gout or psychiatric

conditions, and nursing home residents were excluded. Since this study included a nutritional component, participants with creatinine clearance <30 ml/min, HbA1c >8.0%, food allergies or intolerances, nutrition therapy or precaution feeding, and baseline protein intake exceeding 1 g/kg body weight/day were also excluded.

The intervention was for the duration of 3 months, with a further follow-up of 3 months after the discontinuation of the intervention for a total of 6 months. The intervention groups received either nutrition (Nu) only (an average of 16 g of protein and 3 g of leucine) or nutrition and biweekly 60-min exercise comprising strength, balance, and resistance training (nutrition with exercise [Nu+Ex]). Both the Nu and Nu+Ex groups were recruited from the National University Hospital ambulatory care clinics and community (i.e., senior activity centers and community centers). Due to the COVID-19 lockdown and the research team's movement restrictions, a comparison control group was recruited from the Choa Chu Kang National University Polyclinic, a primary care center located within a housing estate in the Western part of Singapore (Figure 1). A total of 269 participants (Figure 1) were enrolled in the study: 82 in the intervention group (Nu = 59; Nu+Ex = 23) and 187 controls, with complete data at 6 months available for 66 in the intervention group (Nu = 44; Nu+Ex = 22) and 112 controls. The allocations to groups were based on ongoing COVID-19 restrictions, e.g., when no group exercises were permitted, participants received nutrition interventions. Furthermore, due to the pandemic, many participants were hesitant to attend follow-up assessments and hence missed them, contributing to the number of individuals lost to follow-up. Therefore, only participants with complete data were included in the final analysis.

2.2. Procedures

During recruitment, three 24-h dietary recalls (2 weekdays and 1 weekend) were conducted to ensure participants' baseline protein intake was ≤1 g/kg body weight/day. Dietary recall data were coded and input into FoodWorks Premium Edition (Version 10, Xyris Software, Brisbane, Australia, 2019) (23). Similar to FoodWorks, a database of Australian and New Zealand foods, nutritional information for food items from Singapore could be obtained directly from nutrition labels on the food items or local databases, such as the Singapore Health Promotion Board's Energy and Nutrient Composition of Food database (24). Protein intake per body weight was calculated by dividing the mean daily protein intake by body weight.

All participants in the Nu and Nu+Ex groups were provided with leucine-enriched protein supplements, predominantly whey and soy protein combination, in the form of specially created local foods or beverages such as granola, coffee, chicken porridge, or specially manufactured beverage powders that are not available for sale locally. Participants received an average of 16 g of protein and 3 g of leucine daily for 3 months. Once a month, study team members contacted participants to monitor compliance and adverse outcomes and obtain three 24-h dietary recalls (2 weekdays and 1 weekend). Participants in the Nu+Ex group were additionally provided biweekly exercise sessions lasting 60 min, each session comprising strength, balance, and resistance exercises using resistant bands conducted by physiotherapists. The control group only received health education advice. The study lasted for 6 months, with participants being assessed at baseline, 3 months, and 6 months post-baseline.

2.3. Demographics and covariates

A combined interview questionnaire consisting of demographics, chronic diseases, medications, perceived health, physical activity and function, depression, frailty, sarcopenia, falls, cognition, and nutrition was administered by trained study team members. Polypharmacy was defined as consuming ≥5 types of medications daily. The FRAIL scale (Fatigue, Resistance, Aerobic, Illness, and Loss of Weight) was used to assess frailty, where pre-frail was defined as 1–2 with a maximum score of 5 (25, 26). The FRAIL scale is based on a 5-item questionnaire and has been validated in many countries across the world, including Singapore (27). The Rapid Physical Assessment (RAPA) tool was used to assess physical activity (28). Katz's activity of daily living (ADL) scale was used to assess ADL, and Lawton's instrumental activities of daily living (IADL) scale was used to assess IADL (29, 30). Participants were classified as fallers if they experienced one or more falls in the past year (31). The Montreal Cognitive Assessment (MoCA) was used to assess cognition (32). Depression was evaluated using the 15-item Geriatric Depression Scale (GDS-15), where scores >5 indicated depression (33). The Mini Nutritional Assessment Short-Form (MNA-SF) evaluated the risk of malnutrition (34).

2.4. Physical performance

Physical performance measures comprised maximum handgrip strength (HGS), gait speed, and the short physical performance battery (SPPB) test. HGS was measured using the Jamar hand dynamometer on the dominant hand with the elbow flexed at 90° in the seated position. The SPPB includes three components—balance, gait speed, and 5× sit-to-stand (STS)—and is scored out of 12 points (4 points per component). A gait speed of <1.0 m/s was considered slow.

2.5. Body composition

Body mass index (BMI) was calculated by dividing body weight (kg) by height squared (m^2). Body composition was measured using the InBody S10 multi-frequency bioelectrical impedance analyzer (BIA), which included measures of body fat percentage (BF%), fat mass (FM), FFM, ASM, body cell mass, and phase angle. Fat mass index (FMI), fat-free mass index (FFMI), and appendicular skeletal muscle index (ASMI) were obtained by dividing FM, FFM, and ASM by height squared, respectively. Sarcopenia diagnosis was based on the 2019 Asian Working Group for Sarcopenia (AWGS) criteria of gender-specific cutoffs for ASMI and either low HGS (<28 kg for men and <18 kg for women) or low physical performance (35).

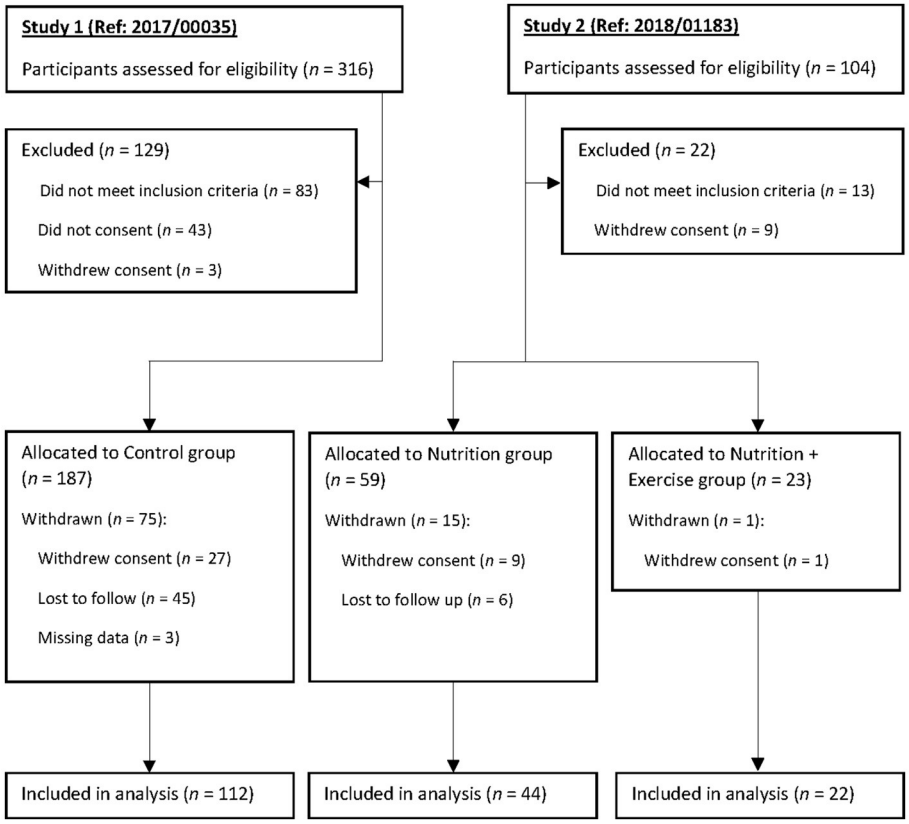

FIGURE 1
Participant screening, group allocation, and follow-up. The per-protocol population consisted of participants who completed the 3-month intervention.

2.6. Inflammatory biomarkers

The TNF-α and IL-6 inflammatory biomarkers were measured by an accredited hospital-based laboratory at 0 and 3 months in a subgroup of participants who agreed to have their blood drawn. TNF-α was measured using immunoenzymetric assays with a detection range between 1.0 and 498 pg/mL, and IL-6 was measured using the electrochemiluminescence immunoassay (ECLIA) with a detection range between 1.5 and 50,000 pg/mL.

2.7. Statistical analysis

IBM SPSS Version 28.0 was used to analyze our data. Per-protocol analysis was performed for participants with a complete set of data. Categorical variables were presented as frequencies with percentages, while continuous variables were presented as mean ± standard deviation when normality assumptions were satisfied (checked using the Shapiro–Wilk test); otherwise, medians with the interquartile range (IQR) were shown. Significance testing of categorical variables was assessed using the chi-square of Fisher's exact tests. Significance testing for normally distributed variables was carried out using a one-way ANOVA; otherwise, the Kruskal–Wallis test was used.

General linear model (GLM) and quantile regression were used to compare changes in normally distributed continuous and non-normally distributed variables between groups, respectively, adjusting for age, sex, ethnicity, years of education, BMI, hypertension, hyperlipidemia, diabetes, polypharmacy, sarcopenia, protein intake, intervention compliance, and corresponding values from the preceding time point.

Plasma biomarker values are presented as median (IQR) in Table 2. Mood's median test was used for significance testing at baseline, while quantile regression was used to compare changes between groups with the control group as the reference. The regression was further adjusted for age, sex, ethnicity, years of education, BMI, hypertension, hyperlipidemia, diabetes, polypharmacy, sarcopenia, protein intake, intervention compliance, and corresponding baseline values.

2.8. Ethics approval and informed consent

This study conformed to the principles of the Declaration of Helsinki and was approved by the National Healthcare Group Domain Specific Review Board (Reference: 2018/01183 and 2019/00017). Informed consent was obtained from all participants.

3. Results

A total of 178 participants were included in the final analysis (Table 1). The mean age of the participants was 72.56 years. There were significantly more males in the control group (55.4%)

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TABLE 1 Baseline characteristics.

	Control	Nutrition	Nutrition + Exercise	*P*-value
	$n = 112$ (62.9%)	$n = 44$ (24.7%)	$n = 22$ (12.4%)	
Demographics				
Age (years)	71.65 ± 5.06	74.57 ± 7.82	71.45 ± 7.07	0.081
Sex				**0.001**
Male	62 (55.4)	12 (27.3)	6 (27.3)	
Female	50 (44.6)	32 (72.7)	16 (72.7)	
Ethnicity				**0.032**
Chinese	92 (82.1)	42 (95.5)	20 (90.9)	
Malay	9 (8.0)	1 (2.3)	0 (0.0)	
Indian	11 (9.8)	1 (2.3)	0 (0.0)	
Others	0 (0.0)	0 (0.0)	1 (4.5)	
Hypertension	81 (72.3)	23 (52.3)	7 (31.8)	**<0.001**
Hyperlipidemia	99 (88.4)	23 (52.3)	8 (36.4)	**<0.001**
Diabetes	60 (53.6)	10 (22.7)	2 (9.1)	**<0.001**
Polypharmacy	32 (28.6)	5 (11.4)	2 (9.1)	**0.018**
Living alone	8 (7.1)	9 (20.5)	3 (13.6)	0.056
BMI (kg/m^2)	26.12 ± 4.51	23.07 ± 3.68	22.73 ± 3.62	**<0.001**
Education (years)	7.45 ± 3.75	7.00 ± 4.34	11.32 ± 6.63	**0.023**
Perceived health (EQ-VAS)	70.01 ± 13.70	68.83 ± 13.21	73.55 ± 12.45	0.404
Physical activity (RAPA)	3.68 ± 1.52	3.05 ± 1.73	3.50 ± 1.37	**0.013**
At least 1 IADL impairment	9 (8.0)	3 (6.8)	1 (4.5)	0.839
At least 1 ADL impairment	19 (17.0)	5 (11.4)	1 (4.5)	0.260
Sarcopenia[a]	14 (12.5)	9 (20.5)	2 (9.1)	**<0.001**
≥1 fall in 1 year	23 (20.5)	18 (40.9)	3 (13.6)	**0.014**
MoCA	25.69 ± 2.86	25.69 ± 3.90	26.19 ± 4.05	0.300
Cognitive impairment	47 (42.0)	17 (38.6)	9 (40.9)	0.930
Depression (GDS)	32 (28.6)	14 (31.8)	7 (31.8)	0.901
MNA-SF total	12.77 ± 1.71	12.50 ± 1.42	12.68 ± 1.09	0.164
Nutritional status (MNA-SF)				0.894
Malnourished	1 (0.9)	0 (0.0)	0 (0.0)	
At risk of malnourishment	19 (17.0)	9 (20.5)	3 (13.6)	
Normal nutritional status	92 (82.1)	35 (79.5)	19 (86.4)	
Physical performance				
Handgrip strength (kg)	23.60 ± 7.02	18.11 ± 5.65	21.16 ± 8.61	**<0.001**
Gait speed (m/s)	0.94 ± 0.20	0.94 ± 0.33	1.18 ± 0.30	**0.003**
5× sit-to-stand time (s)	13.18 ± 5.11	14.25 ± 5.49	10.86 ± 2.35	**0.004**
Total SPPB score	9.86 ± 1.99	8.84 ± 2.51	11.05 ± 1.46	**<0.001**
Body composition				
Body fat percentage (%)	33.62 ± 9.75	32.18 ± 7.69	25.22 ± 5.32	**<0.001**
Fat mass index (kg/m^2)	9.02 ± 3.79	7.58 ± 2.78	6.70 ± 2.95	**0.006**
Fat-free mass index (kg/m^2)	16.89 ± 1.94	15.35 ± 1.78	16.24 ± 2.31	**<0.001**

(Continued)

TABLE 1 (Continued)

	Control	Nutrition	Nutrition + Exercise	*P*-value
Fat mass/fat-free mass	0.54 ± 0.24	0.50 ± 0.17	0.42 ± 0.18	0.096
50 khz trunk phase angle (θ)	5.06 ± 0.65	4.41 ± 0.68	4.78 ± 0.63	**<0.001**
Body cell mass (kg)	27.13 ± 6.28	22.74 ± 4.89	25.16 ± 4.80	**<0.001**
ASMI (kg/m^2)	6.90 ± 1.06	5.76 ± 1.15	6.19 ± 1.12	**<0.001**

Values presented as *n* (%) or mean ± SD. BMI, body mass index; ADL, activities of daily living; MoCA, Montreal Cognitive Assessment; SPPB, short physical performance battery; ASMI, appendicular skeletal muscle index. [a]Based on the Asian Working Group for Sarcopenia (AWGS) 2019's definition. Bold indicates significance ($p < 0.05$).

than in the Nu (27.3%) and Nu+Ex (27.3%) groups. Men were generally reluctant to participate in interventions, which is a known phenomenon worldwide. There was a higher prevalence of the Chinese ethnic group in the Nu group (95.5%), followed by the Nu+Ex group (90.9%) and the control group (82.1%). The Indian and Malay ethnic minority groups were significantly higher in the control group (9.8 and 8.0%, respectively) compared with the Nu (2.3% each) and none in the Nu+Ex group. Diabetes prevalence based on self-reporting was significantly higher in the control group (53.6%) compared with the Nu (22.7%) and Nu+Ex groups (9.1%). This was also evident in the prevalence of hypertension and hyperlipidemia, which was the highest in the control group and the lowest in the Nu+Ex group.

3.1. Physical function, cognition, depression, and perceived health

Baseline physical functions, such as gait speed, HGS, 5× STS, and total SPPB scores, were significantly different between the groups (Table 1). Gait speed was significantly lower in the control (0.94 m/s) and Nu groups (0.94 m/s) compared with the Nu+Ex group (1.18 m/s). The total SPPB score was significantly lower in the Nu group (8.84), followed by the control group (9.86) and the Nu+Ex group (11.05). Similarly, 5× STS time was significantly longer in the Nu group compared with the control and Nu+Ex groups at 14.3 s, 13.2 s, and 10.9 s, respectively. There were no significant differences in perceived health or depression at baseline between the groups.

Within the Nu+Ex group, there was a significant improvement in gait speed at 3 months (β 0.16, 95% CI 0.03–0.28; $p = 0.005$) (Figure 2A), depression as reflected by decreased GDS score (β −1.91, 95% CI −3.20 to −0.62) and improved perceived health (β 7.10, 95% CI 0.42–10.30; $p = 0.036$) (Figure 2B). At 6 months, there was a decline in both the Nu and Nu+Ex groups, with no significant difference between the groups (Figure 2).

Compared with the control group and after adjustment for confounding factors and baseline measures, the Nu+Ex group improved significantly in gait speed (β 0.17, 95% CI 0.01–0.33; $p = 0.040$), 5× STS (β −3.52, 95% CI −6.40 to −0.65; $p = 0.017$), total SPPB scores (β 1.35, 0.19–2.50; $p = 0.023$), GDS scores (β −2.03, 95% CI −3.67 to −0.38) (Figure 3), and perceived health (β 8.35, 95% CI 1.41–12.50; $p = 0.023$) (Supplementary Table 1) at 3 months.

3.2. Body composition

Baseline FFMI, phase angle, body cell mass, and ASMI were significantly higher in the control group (16.89 kg, 5.06 kg, 27.13 kg, and 6.90 kg/m^2) compared with the Nu+Ex (16.24 kg, 4.78 kg, 25.16 kg, and 6.19 kg/m^2) and Nu groups (15.35 kg, 4.41 kg, 22.74 kg, and 5.76 kg/m^2), respectively. Within the Nu+Ex group, there was significant improvement in FFMI (β 0.53, 95% CI 0.01–0.54; $p = 0.029$), ASMI (β 0.19, 95% CI 0.04–0.42; $p = 0.037$), and body cell mass (β 0.66, 95% CI 0.07–1.25; $p = 0.039$) (Figure 2C). At 6 months, there was a decline in FFMI, body cell mass, and appendicular skeletal mass index in the Nu and Nu+Ex groups, with no significant difference between the groups.

Compared with controls and after adjustment for confounding factors and baseline measures, the Nu+Ex group showed significant improvement in the FFMI (β 0.98, 95% CI 0.24–1.72; $p = 0.010$), ASMI (β 0.39, 95% CI 0.06–1.48; $p = 0.020$) and body cell mass (β 1.02, 95% CI 0.22–1.82; $p = 0.013$). The Nu group only showed improvement in body cell mass (β 0.71, 95% CI 0.10–1.32; $p = 0.023$) (Figure 3).

3.3. Inflammatory biomarkers

There were no significant differences in the baseline median levels of IL-6 and TNF-α between the groups (Table 2). However, at 3 months, there was a significant decrease in the IL-6 levels in the Nu+Ex group (β −4.32, 95% CI −5.31 to −3.05; $p < 0.001$) and the Nu group (β −8.24, 95% CI −9.05 to −7.19; $p < 0.001$) (Table 2). Similarly, there was a significant decrease in the TNF-α levels at 3 months in the Nu+Ex (β −2.10, 95% CI −3.26 to −1.01; $p < 0.001$) and Nu groups (β −1.88, 95% CI −2.94 to −0.81; $p < 0.001$).

4. Discussion

Multidomain interventions incorporating exercise and nutrition have been shown to reverse frailty at the population level and are an important public health priority in many countries (4, 5, 36). Our study demonstrated that 3 months of leucine-enriched protein supplementation and exercise intervention in pre-frail older adults who consumed ≤1 g/kg of protein were associated with significant improvement in physical function measures such as gait speed, SPPB, and 5× STS, depression, perceived health, and body composition measures such as FFMI, ASMI, and body cell mass. These were further supported by improvements in inflammatory biomarkers. The Nu group had

significant improvements only in body cell mass and inflammatory biomarkers but non-significant improvements in physical function and ASMI. The improvements were not sustained after the discontinuation of the interventions, which suggests a significant contribution of the interventions to the outcomes, especially in the Nu+Ex group. The intake of protein in older adults is often suboptimal, as shown by the 2005–2014 National Health and Nutrition Examination Survey, where 46% of the oldest adults did not meet the requirement of 0.8 g/kg per day (37). Studies have shown that in older adults above 70 years old, lower than 0.8 g/kg per day of protein intake was associated with greater functional limitation and lower HGS (37). Between 2013 and 2014, both the PROT-AGE Study Group and the European Society for Clinical Nutrition and Metabolism (ESPEN) Group recommended increased protein intake of 1.0–1.2 g/kg of body weight per day or higher in older adults, and countries like Singapore have recommended an increase in the RDA of protein for older adults to 1.2 g/kg/day (10, 38, 39).

The amount, quality, type, and timing of protein supplements are known to affect absorption, digestion, and availability. Proteins can be categorized as "fast," such as whey or soy protein, or slow, such as casein, depending on the speed of release of amino acids. There has been increasing interest in recent years in the use of "fast" proteins to counteract anabolic resistance and improve muscle protein synthesis and muscle function. "Fast" protein in combination with exercise and/or omega-3 fatty acids has been shown to stimulate muscle protein synthesis (40). Studies have shown that peak protein synthesis happens 2 h post-meal, and until recently, the recommendations have been to distribute protein intake across the meals, such as 0.4/kg/meal or 30 g/meal, to counteract anabolic resistance and limit muscle mass loss (41–43). Leucine is recognized as an anabolic stimulus and together with exercise acts through the mechanistic target of the rapamycin complex 1 (mTORC1) pathway in enhancing muscle protein synthesisand immunomodulatory function and reducing muscle protein breakdown (44, 45). Participants in our study received leucine-enriched whey and soy protein, and while we have no information on the timings of leucine-enriched protein supplementation, participants in the Nu+Ex group showed improvement in physical function, inflammation, and body composition measures. Unlike other studies that targeted sarcopenic older adults and supplemented whey protein with vitamin D, leucine-enriched protein supplementation alone did not result in a significant improvement in physical function in our study population (46, 47). The differences between studies could be due to varied study methodology, timing and type of protein intake, type and intensity of exercise, study population, and duration of follow-up (48).

Anabolic resistance with aging is possibly explained by multiple interacting factors such as altered gut microbiome affecting absorption, increased splanchnic sequestration of amino acids affecting peripheral availability, decreased muscle amino acid

FIGURE 2 (Continued)

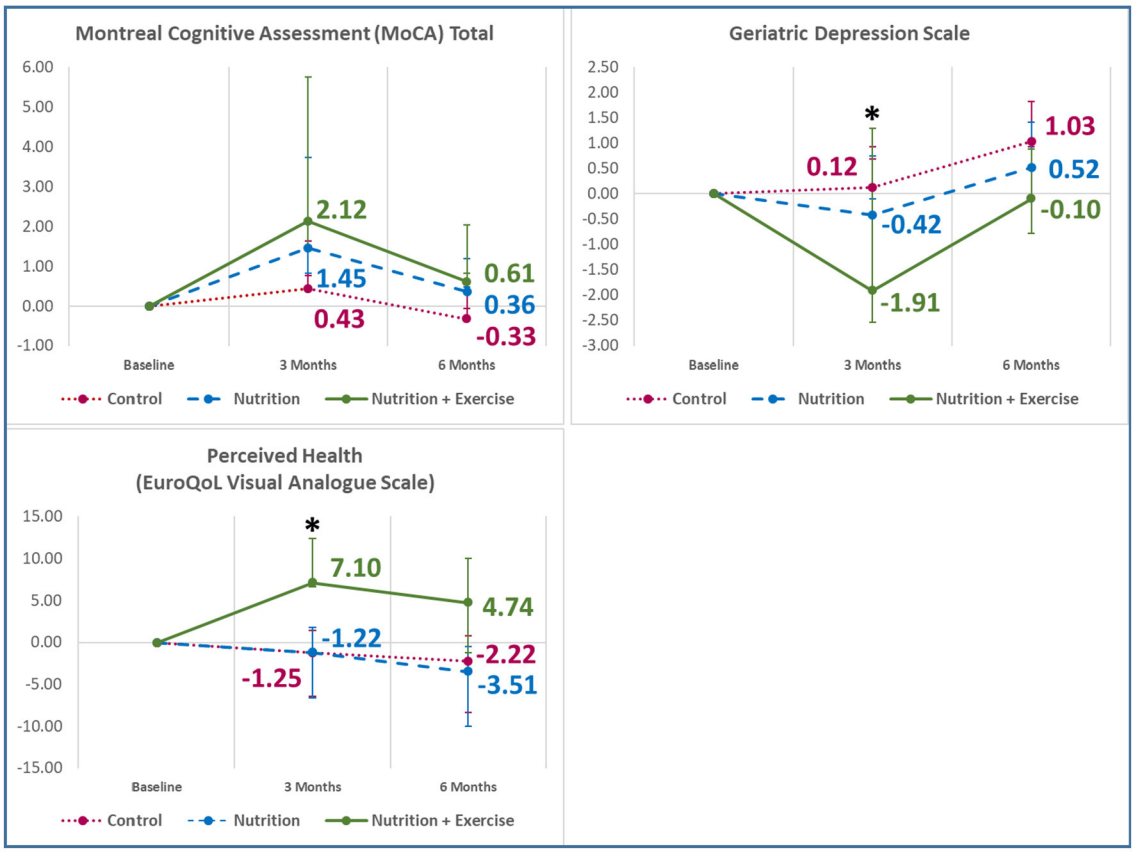

FIGURE 2 (Continued)

uptake, lower postprandial perfusion of muscle, and dysfunctional muscle protein synthesis signaling (16). Exercise has been shown to improve the sensitivity of skeletal muscle to dietary protein, and studies have shown that a combination of higher dietary protein and resistance exercise is required for muscle protein synthesis and to counteract anabolic resistance with aging (16). Participants in the Nu+Ex group improved significantly in physical function and muscle mass indices at 3 months.

The benefits of exercise are postulated to be mediated through the activation of the mTORC1 pathway and the release of myokines, osteokines, adipokines, and immune cytokines. The systematic reviews on the benefits of nutrition in addition to exercise have shown mixed results due to varied target groups and nutrition supplements (49). Rondanelli et al. showed that 12 weeks of supplementation with 22 g of whey protein, amino acids including 4 g of leucine, and vitamin D, together with controlled physical activity, increased FFM, physical strength and function, quality of life, and reduced malnutrition in sarcopenic older adults (50). A recent study by Oh et al. showed a leucine-rich protein supplement (20 g protein with 2 g of leucine) together with resistance exercise in healthy older adults improved muscle mass at 12 weeks (11). Recently, a meta-analysis by Negm et al. showed that mixed exercises were most effective in improving muscle mass, whereas physical activity, protein supplementation, and aerobic exercise were most effective in improving physical performance in sarcopenic individuals (51). While the benefit of combined interventions is evident in sarcopenic individuals, studies on the effectiveness of combined interventions in pre-frail or frail participants show mixed results (46, 47, 50, 52). Our study participants participated in mixed exercises biweekly, and the Nu+Ex group showed significant improvement in physical function, FFMI, and ASMI, which was not evident in the control or Nu groups. Various studies have shown that whey protein supplementations of between 22 and 25 g and leucine between 3 and 4 g with vitamin D in combination with exercise intervention increased FFM, relative skeletal muscle mass, and physical function in sarcopenic older adults (50). Pre-frail participants in the Nu+Ex group and Nu group improved in body cell mass. Body cell mass is increasingly recognized as a reliable indicator of muscle mass loss, a measure of metabolically active tissue mass, and nutrition, and is significantly associated with whole body phase angle, which is a significant predictor of mortality (50, 53).

Participants in the Nu+Ex group improved significantly in their GDS scores and perceived health. Berens et al. also showed similar findings from the Vigor 2 study, where combined nutrition and physical activity, but not the nutrition intervention or placebo arm, improved both depression and health-related quality of life (HRQOL) (54). Another study similarly showed physical exercise training and nutritional intervention, but not exercise training alone, over 12 weeks in pre-frail women had beneficial effects on several HRQOL domains (55). Elevated IL-6 has been implicated in depression symptom severity, and 12 weeks of once-weekly exercise

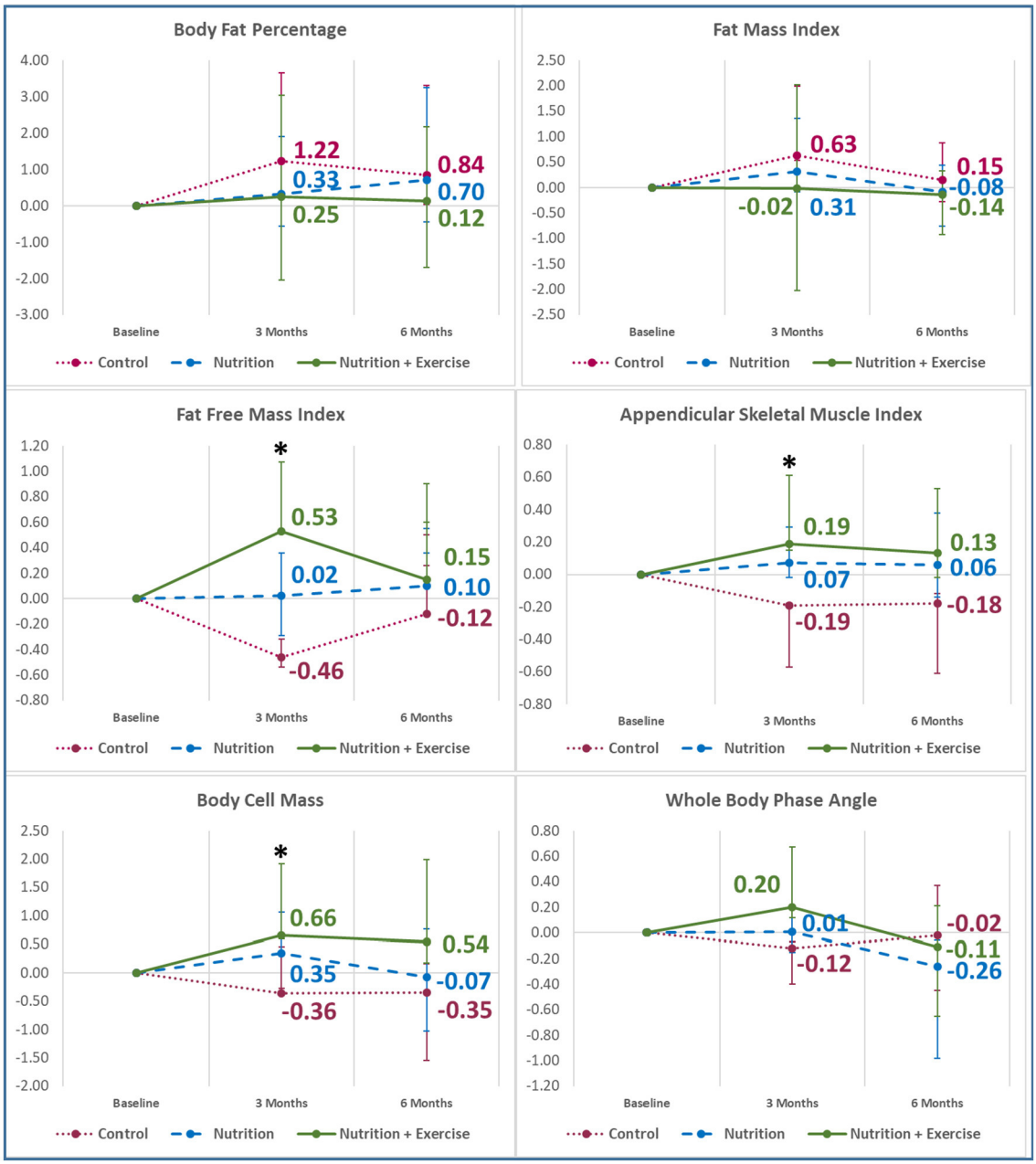

FIGURE 2 (Continued)

sessions have shown a concurrent reduction of depression severity and IL-6 (56). Studies have attributed improvements in depression to exercise due to possible mediation through myokines such as irisin or amino acids like tryptophan which have an antidepressant-like effect (57, 58). It is not known if the improvement in depression in our study participants was mediated through a reduction in IL-6 or through other mechanisms.

Amino acids play a significant role in the proliferation and activation of lymphocytes, natural killer cells, macrophages, and the production of antibodies and cytokines (59). Insufficient dietary protein or amino acid intake has a major impact on the immune system, increases susceptibility to infectious disease, and may exacerbate low-grade inflammation associated with aging (60). Inflammaging, defined as chronic low-grade inflammation with aging, has been associated with many negative outcomes, including insulin resistance, cardiovascular disease, and mortality (60). Most previous studies on the association of protein supplementation with proinflammatory cytokines have focused on healthy older adults, diabetics, or sarcopenic obesity, where the impact on IL-6 and TNF-α has been variable (17, 61, 62). There are as yet no studies on the impact of leucine-enriched protein supplements on IL-6 and TNF-α in pre-frail older adults. Participants in the Nu+Ex and Nu groups showed significant reductions in IL-6 and TNF-α further supporting the role of amino acids in inflammation. Inflammation is common in aging, frailty, and sarcopenia, and it is not known if the improvement in inflammatory cytokines could be mediated

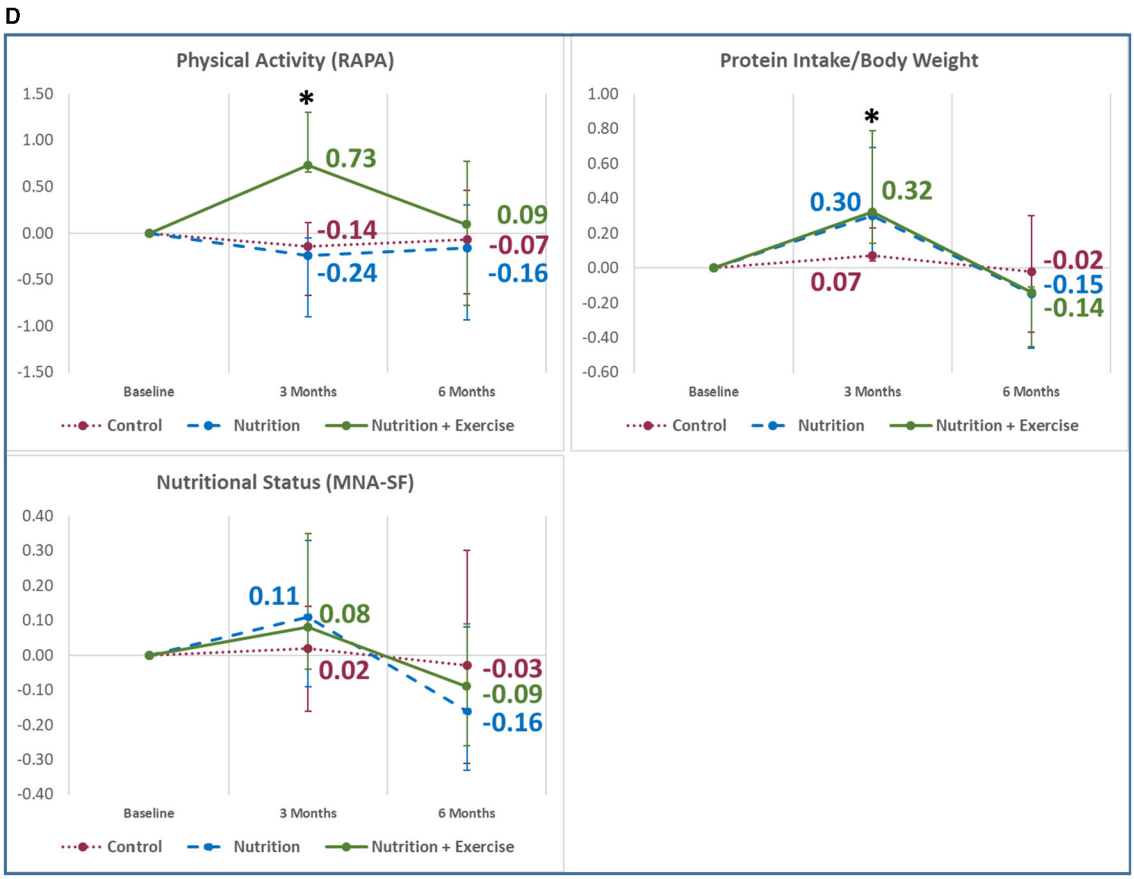

FIGURE 2 (Continued)
Mean changes in outcome variables from baseline to 6 months in **(A)** physical function; **(B)** cognition, depression, and perceived health; **(C)** body composition; **(D)** physical activity and nutritional information. Analysis adjusted for age, sex, ethnicity, education, body mass index, hypertension, hyperlipidemia, diabetes, polypharmacy, sarcopenia, protein intake, intervention compliance, and corresponding values from the preceding time point. Vertical bars indicate the 95% confidence interval. *$p < 0.05$; **$p < 0.001$; RAPA, rapid assessment of physical activity; MNA-SF, Mini Nutritional Assessment Short-Form.

through improvements in body composition parameters such as muscle mass or through other mechanisms.

The main strength of this study was robust physical assessments and compliance checking. However, there are several limitations that warrant mention. First, one of the major limitations was the lack of randomization and the significant differences between the control and intervention groups, which were adjusted for in the final analysis. The study was conducted during the COVID-19 pandemic, which interrupted some of the follow-ups and interventions, resulting in variable sample sizes and differences between study populations. Despite the small sample size, significant correlations were obtained, and our results hold clinical value, which requires further validation. Second, chronic disease, 24-h dietary recalls, and functional ability were based on self-reporting, which may be subject to recall bias. Third, there was higher reporting of chronic diseases in the control group, but they had better functional and body composition measures and similar baseline inflammatory biomarkers. This could be due to a recent visit to the doctor for a follow-up on their chronic diseases. The results were adjusted for baseline differences, and benefits were seen only during the intervention period, suggesting a significant contribution of leucine-enriched protein and exercise in the improvement of functional parameters and body composition measures. Fourth, we did not have information on the intake of supplements such as vitamin D and omega-3, which may impact muscle protein synthesis. Fifth, although we advised participants to spread out their protein intake between meals, we have no information on timing, other proteins in daily meals, or the distribution of protein consumption. Our participants mainly consumed a combination of leucine-enriched soy and whey protein. Studies have shown that both are effective in muscle protein synthesis, with no significant differences in strength or muscle mass gain between leucine-enriched soy or whey protein in combination with resistance exercise intervention after 12 weeks (63–65). Sixth, we have no comparison with the exercise-only arm and therefore find it difficult to determine whether the improvements in the Nu+Ex group were due to just the exercise or an interaction between exercise and nutritional supplementation. However, improvement in inflammatory biomarkers was also seen in the Nu group, suggesting a leucine-enriched diet may have a complementary effect. Lastly, due to multiple interacting effects and our study being a quasi-experimental non-randomized study,

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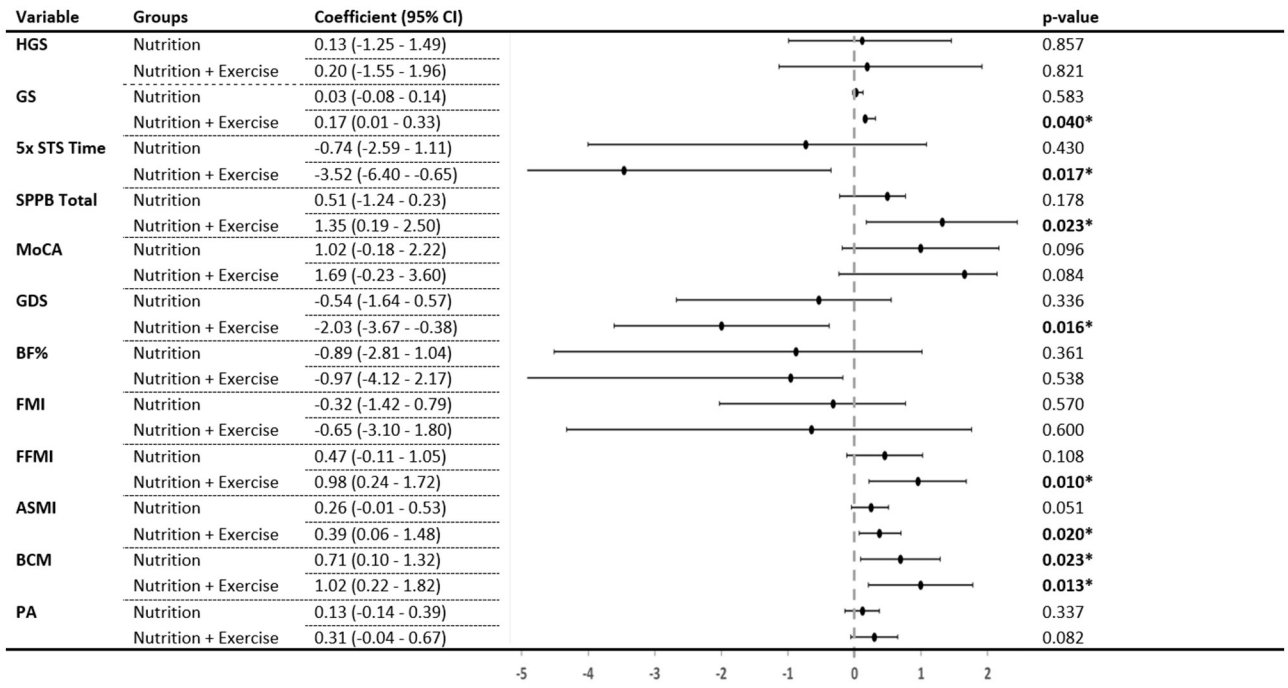

FIGURE 3
Change in each group compared with the control group after 3 months. *p*-values generated from a general linear model with age, sex, ethnicity, education, body mass index, hypertension, hyperlipidemia, diabetes, polypharmacy, sarcopenia, protein intake, intervention compliance, and corresponding values from the preceding time point as covariates. HGS, handgrip strength; GS, gait speed; 5× STS, 5× sit-to-stand; SPPB, short physical performance battery; MoCA, Montreal Cognitive Assessment; GDS, Geriatric Depression Scale; BF%, body fat percentage; FMI, fat mass index; FFMI, fat-free mass index; ASMI, appendicular skeletal muscle index; BCM, body cell mass; PA, whole-body phase angle. *Indicates significant difference ($p < 0.05$) when compared with the control group.

TABLE 2 Unadjusted and adjusted quantile regression models of median change in plasma biomarkers after 3 months of intervention.

Biomarker	Group	Baseline median (IQR)[#]	Unadjusted	Adjusted[+]
			Coefficient (95% CI) *p*-value	Coefficient (95% CI) *p*-value
IL-6 (pg/mL)	Control	3.20 (1.80)	Reference	Reference
	Nutrition	2.80 (1.20)	−5.10 (−1.69 to 0.67) $p = 0.383$	**−8.24 (−9.05 to −7.19) $p < 0.001$**
	Nutrition + Exercise	2.30 (0.80)	−0.20 (−1.14 to 0.75) $p = 0.672$	**−4.32 (−5.31 to −3.05) $p < 0.001$**
TNF-α (pg/mL)	Control	8.30 (4.80)	Reference	Reference
	Nutrition	8.10 (5.30)	**−2.10 (−3.79 to −0.41) $p = 0.017$**	**−1.88 (−2.94 to −0.81) $p < 0.001$**
	Nutrition + Exercise	6.50 (1.40)	**−2.30 (−3.58 to −1.02) $p < 0.001$**	**−2.10 (−3.26 to −1.01) $p < 0.001$**

Biomarker data were available for 51 participants (Control, $n = 20$; Nutrition, $n = 18$; Nutrition + Exercise, $n = 13$). [#]No significant difference ($p > 0.05$) between medians at baseline; [+]Adjusted for age, sex, ethnicity, education, body mass index, hypertension, hyperlipidemia, diabetes, polypharmacy, sarcopenia, protein intake, intervention compliance, and corresponding baseline values. IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha. Bold indicates significance ($p < 0.05$).

causal inferences cannot be assumed, and our findings need to be validated in a larger prospective randomized trial. We did not evaluate the palatability and acceptability of the nutrition supplement.

Collectively, data from previous studies in healthy and sarcopenic older adults and our exploratory analysis in pre-frail older adults suggest that leucine-enriched protein supplementation and exercise combination improve physical function, depression, perceived health, body composition such as FFM and body cell mass, and overall inflammation as evident by reduced IL-6 and TNF-α (11, 18, 46–48, 50, 62). To the best of our knowledge, there are limited studies evaluating the benefits of leucine-enriched protein and exercise in terms of physical function, body composition, and inflammation biomarkers in pre-frail older adults with suboptimal baseline protein intake. Our study findings need to be revalidated in future prospective randomized trials targeting at-risk individuals with insufficient protein intake.

Our study demonstrated that additional protein and leucine supplementation together with biweekly exercise over 3 months in pre-frail older adults who consumed ≤1 g/kg of protein per day was associated with improvement in gait speed, SPPB, 5× STS, depression, FFMI, and body cell mass on body composition measures and inflammation.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by National Healthcare Group Domain Specific Review Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

RM obtained funding. RM, EP, and KB designed the study. RM, DA, and YC contributed to data analysis and drafting of charts and tables. RM, DA, AL, VN, and YC contributed to the drafting of the manuscript. All authors read, edited, and approved the version submitted.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1204198/full#supplementary-material

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Morbidity and mortality related to type II odontoid fractures in octogenarians undergoing surgery: a retrospective study with 5 year follow up

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Introduction: The prevalence of trauma is increasing in the geriatric population. The optimal therapy for type II odontoid fractures in the elderly is controversial. This study aims to assess the morbidity and mortality associated with odontoid fractures in octogenarians undergoing C1/C2 posterior screw fixation and describe the perioperative and post-operative complications and risk factors associated with mortality.

Materials and methods: Electronic medical records from a single institution pertaining to the period between September 2005 and December 2020 were retrieved. Data on patient demographics, neurological conditions, surgical characteristics, complications, hospital course, and 90-day mortality were collected.

Results: Over a 16-year period, 60 patients aged ≥80 years diagnosed with type II odontoid fractures were enrolled in the study. The mean age was 85.0 ± 1.9 years. The mean Charlson Comorbidity Index (CCI) was >6 indicating a poor baseline reserve (8.5 ± 1.9), while cardiovascular diseases were the most prevalent among comorbidities. The mean surgical duration was 217.5 ± 65.9 min, with a mean blood loss of 725.5 ± 275.7 mL. The in-hospital was 5–0% and the 90-day mortality rates increased at 10.0%. No revision surgery was needed in any of the cases. Intraoperative and post-operative X-ray and computed tomography (CT) imaging revealed correct screw placement. Proper alignment of the atlantoaxial spine and fusion could be achieved in all cases. The unique risk factors for mortality included the presence of comorbidities and the occurrence of post-operative complications.

Conclusion: The complication and mortality rates associated with odontoid fractures in octogenarians are relatively high. However, the therapeutic goals in this population also include bone union and preservation of neurological status. Despite the often-high comorbidity rate, we still recommend that surgery should be considered in patients over 80 years. However, it is necessary to evaluate several approaches when treating such frail patients.

KEYWORDS

odontoid fractures, low energy trauma, octogenarians, fusion, comorbidities

1. Introduction

With the global trend of increasing life expectancy, healthcare systems worldwide are confronted with the medical needs of older patients. Thus, medical/surgical strategies need to be adjusted to the unique needs of this steadily evolving population. For instance, geriatric trauma continues to increase in prevalence, and it is predicted that by the year 2050, nearly 40% of all injured patients will be aged >65 years. In terms of spine fractures, odontoid fractures constitute the most common fracture of the axis, and the most prevalent type of fracture of the cervical spine among patients aged 65 years and older (1, 2).

The susceptibility of older patients to such fractures might be attributed to the loss of bone mineral density due to the increased incidence of osteoporosis or to degenerative disorders causing the calcification of ligaments; these conditions result in a decreased ability to absorb traumatic impact. Consequently, a low velocity trauma such as a fall from a standing posture can cause such fractures (3).

In spite of the high prevalence of odontoid fractures among the elderly (range 2.4–4.7%) (4), its treatment remains controversial. Previous studies suggest that conservative management such as halo vest immobilization or rigid cervical orthosis lead to decreased rates of fusion and contribute to higher rates of chronic pain, neurological deterioration, morbidity, and mortality (5, 6). In contrast, surgical management such as C1/C2 posterior screw fixation has been reported to produce promising results with high rates of biomechanical stability and fusion compared to conservative management (7, 8). However, a major drawback of surgical techniques is, due to the poor baseline reserve in the elderly, that the surgical risks are high in this population (9). Striking the right balance between risk and benefit and formulating the right therapeutic strategy remains a difficult proposition.

To address this issue, the present study seeks to describe the morbidity and mortality rates in octogenarians with odontoid fractures undergoing C1/C2 posterior screw fixation and assess the perioperative and post-operative complications and risk factors associated with mortality.

2. Materials and methods

2.1. Study design and patient characteristics

Clinical and imaging data were retrospectively collected for the period between September 2005 and December 2020 from our institution's database. This study was approved by the local ethics committee of our institution (approval number 880/2021) and was conducted in accordance with the Declaration of Helsinki. The requirement for informed consent was waived due to the retrospective nature of the study.

Patients aged ≥80 years with type II odontoid fractures diagnosed on both cervical spine radiograph and computed tomography (CT) images were included (**Figure 1**). A CT-angiography was performed to examine the presence of anatomical abnormalities or kinking in the course of the vertebral artery before surgery. Magnetic resonance imaging (MRI) of the cervical spine was performed to evaluate the integrity of the spinal ligaments. The exclusion criteria were as follows: congenital instability, rheumatoid arthritis, instability caused by a tumor, spinal infections, and previous cervical surgery. The German guidelines for trauma mechanisms were used to define low energy trauma (LET) and the patients' injuries were classified accordingly (10). LET was defined as a fall from a sitting or standing position or a low height (<1 m) (10).

2.2. Outcome parameters

Data regarding patient demographics, comorbidities, American Society of Anesthesiologists scores, surgical duration, number of treated spinal levels, perioperative and post-operative complications, length of hospital stay, intensive care unit (ICU) stay, readmission, reoperation, and mortality were retrieved from the patients' electronic records. Comorbidities present preoperatively were assessed using the age-adjusted Charlson Comorbidity Index (CCI). The CCI was calculated for each patient and classified as no comorbidity, minimal comorbidity, moderate comorbidity, or severe comorbidity (CCI of 0, 1 or 2, 3 to 5, and >5, respectively) (11, 12). The pre-treatment neurological condition was assessed using the American Spinal Injury Association impairment scale (AIS A: complete sensory and motor loss, B: sensory loss incomplete but complete motor loss, C: motor loss incomplete with key muscle test less than a grade of 3; D: motor loss incomplete with key muscle test more than a grade of 3; E: complete return of all motor and sensory function) (13). Post-treatment AIS data were obtained from the last documented clinical encounter. Routine clinical and radiological follow-up examinations were performed before discharge and 3 months after surgery according to our institutional standards. The follow-up period was between 3 and 72 months after surgery. Conventional radiographs in the anteroposterior and lateral views were obtained to evaluate the screw position and fusion rates.

2.2.1. Decision process

Patients aged ≥80 years with an acute unstable odontoid fracture type II were enrolled in the study. The diagnosis of acute odontoid fracture was based on a thin-slice computed tomography (CT) scan. The decision making was jointly guided by both clinical and radiological parameters. In cooperation with experienced neuroradiologists, meticulous assessments of the CT images and following predictors were considered, as previously recommended (14–16): non-union displacement >4 mm, posterior displacement, age of trauma, and fracture coagulation of more than 10°.

2.3. Surgical technique

A modified Goel-Harms technique in conjunction with a posterior arch C1 lateral mass screw (PALM) was used for the C1/C2 posterior screw fixation (17, 18). By applying this technique, the risk of bleeding from the epidural venous plexus as well as postsurgical neuralgia resulting from the manipulation of the C2 nerve root can be mitigated as the posterior arch of C1 is used as an entry point. C2 pedicle screws were inserted according

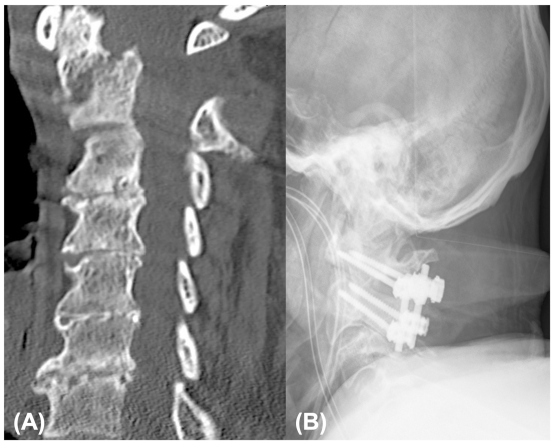

FIGURE 1
A total of 85 year male patient presenting with arm monoparesis: emergency CT showing type II odontoid fracture with anterior displacement **(A)**, post-operative anterior-posterior radiographs displaying posterior screw fixation of C1/C2 **(B)**.

to Harms' technique in most cases (19). In some cases, the C2 screws also were inserted according to Harms' technique in the pars of C2, lateral to the superior margin of the C2 lamina (19). All instrumented surgeries were performed using a CT-based point-to-point navigation system to maximize safety, as previously described by our study group (20). All instrumented surgeries were performed using a CT-based point-to-point navigation system to maximize safety, as previously described by our study group (20). After surgery, none of the patients were kept in a cervical collar, as recommended by the German Guidelines of the spine community for the management of cervical fractures encompassing treatment approaches for odontoid type II fractures (21).

2.3.1. Post-operative care and monitoring

Following the surgical procedure, all patients were transferred to the Intensive Care Unit (ICU) for close monitoring. This standard protocol was adhered to ensure optimal post-operative care, allowing for the immediate detection and management of any potential complications. Each patient remained in the ICU for a minimum duration of 1 day, with the exact duration determined by the attending physician's assessment of the patient's recovery and stability.

2.4. Statistical analysis

Categorical variables are presented as numbers and percentages. Continuous variables are presented as means ± standard deviations; the Shapiro–Wilk test was used to verify whether the data distribution was normal. Baseline characteristics, duration of surgery, number of treated spinal levels, perioperative and post-operative complications, length of stay (LOS), ICU stay, readmissions, reoperations, and mortality were compared groupwise using independent *t*-tests for continuous variables and chi-squared tests for categorical variables. The chi square test was used to evaluate changes in neurological status (AIS). In the second-stage analysis, a binary logistic regression analysis was performed to identify the risk factors for mortality. Statistical significance was set at a *p*-value of 0.05 or less.

3. Results

3.1. Patient demographics and baseline characteristics

Sixty patients aged ≥80 years diagnosed with type II odontoid fractures were enrolled in the study over a 16-year period. The mean age was 85.0 ± 1.9 years; there was a predominance of female patients (n = 38, 63.3%) among the study population. The mean CCI was >6, indicating a poor baseline reserve (8.5 ± 1.9). The prevalence of arterial hypertension, coronary heart disease, and atrial fibrillation was high (n = 53, 88.3%; n = 37, 61.7%; n = 27, 45.0%, respectively). The mean MS was 86.9 ± 20.1, indicating the presence of a new motor deficit. A detailed breakdown of the patient characteristics is presented in Table 1.

3.2. Surgical characteristics

As shown in Table 2, the mean surgical duration was 217.5 ± 65.9 min, with a mean blood loss volume of 725.5 ± 275.7 mL. The mean number of decompressed levels was 1.7 ± 0.8. The mean ICU stay was 3.9 ± 0.3 days, while the hospital stay lasted 13.0 ± 8.5 days. During the hospital stay, three patients (5.0%) died from septic events, while another three (5.0%) died 3 months after surgery due to cardiovascular disease. No further surgeries were required during the follow-up period. The AIS improved significantly after surgery ($p < 0.001$). Overall, the mean follow-up period was 67.3 ± 17.7 months, and no additional surgery to treat secondary instability was necessary. Intraoperative and post-operative radiographic and CT imaging revealed correct screw placement. Proper alignment of the atlantoaxial spine could be achieved in all cases. Fusion could be achieved in all patients by clearly visible, continuous bony trabeculation as evaluated by radiographic imaging at the final follow-up. During the follow up period, three additional patients deceased due to an acute heart failure, while no patients died due to postsurgical complications. The overall mortality rate increased to 15.0% (n = 9/60).

3.3. Occurrence of adverse events and potential risk factors

The most prevalent complications were pneumonia (20.0%), followed by urinary tract infection (8.3%), acute heart failure (6.7%), pleural effusion (6.7%), and septic events (5%). Detailed data regarding post-operative complications are presented in Table 3. In the second stage analysis, we investigated the potential risk factors for mortality. The presence of comorbidities and post-operative complications were unique risk factors for mortality; surgical duration, estimated blood loss, and hospital or ICU stay were not risk factors for mortality (Table 4). Separate analyses were performed to evaluate potential collinearity between variables of

TABLE 1 Baseline characteristics.

Characteristic	Value
Number of patients	60
Age, years (mean, SD)	85.0 (1.9)
Sex (*n*, %)	
Male	22 (36.7)
Female	38 (63.3)
Body mass index, kg/m^2 (mean, SD)	30.8 (5.1)
Comorbidities	
Age-adjusted CCI score (mean, SD)	8.5 (1.9)
Arterial hypertension (*n*, %)	53 (88.3)
Myocardial infarction (*n*, %)	24 (40.0)
Coronary heart disease (*n*, %)	37 (61.7)
Atrial fibrillation (*n*, %)	27 (45.0)
Heart failure (*n*, %)	20 (33.3)
COPD (*n*, %)	10 (16.7)
Diabetes mellitus type II (*n*, %)	15 (25.0)
Renal failure (*n*, %)	13 (21.7)
Liver disease (*n*, %)	4 (6.7)
Gastrointestinal ulcer (*n*, %)	14 (23.3)
TIA/stroke (*n*, %)	13 (21.7)
Malignancy (*n*, %)	14 (23.3)
Dementia (*n*, %)	17 (28.3)
Previous spinal surgery (*n*, %)	3 (5.0)
ASA class (*n*, %)	
II	16 (26.6)
III	38 (63.3)
IV	5 (8.3)
Preoperative MS (mean, SD)	86.9 (20.1)

ASA, American society of anesthesiologists; CCI, Charlson comorbidity index; COPD, chronic obstructive pulmonary disease; MS, motor score of the American spinal injury association grading system; SD, standard deviation; TIA, transient ischemic attack.

interest. When hospital stay and ICU stay, as well as the duration of surgery and blood loss, were individually introduced into the multivariate model, the outcomes remained consistent with the primary results.

4. Discussion

Cervical spine fractures are associated with high morbidity and mortality rates in the geriatric population (22–24). Odontoid fractures are highly prevalent among the elderly. Due to degenerative and osteoporotic alteration of the dens axis in this population, minor trauma such as a fall from a standing position can cause such fractures. While odontoid fractures are relatively frequent, their therapeutic management is still a subject of debate; both operative and non-operative treatment options are associated with several disadvantages. It is important to note that the existing evidence is predicated upon a small retrospective series and may not help in resolving the dilemma of whether or not to treat an octogenarian patient with an odontoid fracture.

TABLE 2 Perioperative and post-operative surgical characteristics and clinical course of 60 patients who underwent C1–C2 posterior screw fixation.

Characteristic	Value
Surgical duration, min	217.5 (65.9)
Estimated blood loss, mL	725.5 (275.7)
Blood transfusion (*n*, %)	16 (26.7)
Hospital stay, days	13.0 (8.5)
ICU stay, days	3.9 (0.3)
Mortality	
In-hospital (*n*, %)	3 (5.0)
90-day (*n*, %)	3 (5.0)
30-day readmission (*n*, %)	0 (0.0)
Post-operative MS	93.9 (15.9)

Values represent the mean (SD) except where otherwise indicated.
ICU, intensive care unit; MS, motor score of the American spinal injury association grading system; SD, standard deviation.

TABLE 3 Occurrence of adverse events in octogenarian patients (*N* = 60) who underwent decompression surgery.

Event	Number of patients (%)
Deep wound infection	1 (1.7)
Acute heart failure	4 (6.7)
Pulmonary embolism	2 (3.3)
Pneumonia	12 (20.0)
Sepsis	3 (5.0)
Pleural effusion	4 (6.7)
Ileus	2 (3.3)
Urinary tract infection	5 (8.3)
Dysphagia	1 (1.7)

TABLE 4 Risk factors associated with mortality.

Risk factor	OR (95% CI)	*p*-value
Age-adjusted CCI score	1.4 (1.1–3.7)	**0.004**
Preoperative MS	0.9 (0.8–1.0)	0.433
Duration of surgery	1.1 (1.0–1.5)	0.590
Estimated blood loss	1.0 (0.9–1.1)	0.696
Length of ICU stay	0.8 (0.6–1.2)	0.308
Length of hospital stay	1.2 (0.8–1.7)	0.055
Complications	2.2 (1.5–3.7)	**0.006**

CCI, Charlson comorbidity index; CI, confidence interval; ICU, intensive care unit; MS, motor score of the American spinal injury association grading system; OR, odds ratio. Bold values indicate statistically significant results.

4.1. Summary of results

To the best of our knowledge, this is the largest retrospective series examining the clinical course of surgical treatment for odontoid fractures in patients ages 80 years and older. We assessed the mortality and morbidity rates and determined the risk factors for both mortality and loss of ambulation exclusively among octogenarians undergoing C1/C2 posterior screw fixation in less

than 72 h after trauma. Our results revealed that octogenarians presented with a poor baseline history, as indicated by a CCI > 6, with cardiovascular diseases being the most prevalent. Both the in-hospital and 90-day mortality rates were at 5.0%. It is important to emphasize that the cause of death was not surgery-related, but due to events unrelated to the surgical procedure. Of note, the neurological condition, as defined by the AIS, improved significantly after surgery. Most importantly, the presence of comorbidities as well as post-operative complications were unique significant predictors of mortality.

4.2. Review of literature

4.2.1. Comorbidities, complications, and clinical course

Performing surgical procedures in older patients for cardiac, orthopedic, or vascular diseases is currently widely accepted, while surgery for traumatic fractures such as odontoid fractures is not yet commonly practiced in the same patient cohort (25). Rivzi et al. reported on a retrospective series of 336 patients with odontoid fractures and analyzed the impact of age and comorbidities on surgical decision-making. They found that preexisting major medical comorbidities were associated with increasing age and a higher level of dependency (26). Note worthily, surgery was mainly preferred in patients with low comorbidity rates as well as independent living, as such patients were more compliant to surgery than their older counterparts. Schoenefeld et al. also showed that surgery was associated with reduced mortality in patients aged between 65 and 74 years, while this association diminished with increasing age; this finding was mainly attributed to the poor baseline reserve of the elderly (27). Issa et al. reported on another retrospective series of nonagenarians undergoing C1/C2 posterior screw fixation due to odontoid fractures, and also found high comorbidity rates; in that study, 80% of the patients were found to have a high risk of perioperative complications (28). In the present study, the rates of comorbidities were high, with a mean CCI of 8.5, indicating poor baseline medical conditions. Nevertheless, each patient underwent emergent surgery due to high grades of dislocation of the dens axis as well as progressive neurological deficit. Emphasizing our findings, age alone should not overshadow clinical necessities when determining the surgical intervention's appropriateness.

It is often debated whether odontoid fractures should be treated surgically due to the related post-operative complications. Charles et al. in their retrospective study on 204 patients with odontoid fractures reported a mortality rate of 12.7% within 1 year, while the rate for patients aged 70 years and older were significantly higher, at 16.7% (29). It is worth noting that the significant predictors of mortality were increasing age and the present of comorbidities, while the treatment choice (surgery vs. conservative) did not significantly impact the outcome (29); these findings are in line with those from the present study. In another review and meta-analysis examining patients with odontoid fractures undergoing surgery, the overall mortality rate was 10.1%; the in-hospital mortality was 6.2% and the 6- and 12-year mortality rates were at 7.4% (9). The study group postulated that increasing age and underlying diseases were the key factors related to post-operative mortality (in agreement with Charles et al.). Overall, they suggested that surgery and posterior screw fixation might be beneficial for geriatric patients in terms of pain relief and occurrence of post-operative adverse events. However, a major limitation of the study is that the study population consisted of individuals aged 65 years and older and long-term follow up data were not available (9); this lowered the significance of their results, and particularly their extrapolation of the findings to octogenarian patient. Similar to the aforementioned studies, Smith et al. performed a retrospective analysis of 32 octogenarians undergoing conservative or surgical management and reported a mortality rate of 12.5% in the surgically treated and of 15% in the conservatively treated cohort, (difference not significant) (7). This study group also stated that patient baseline history and age were associated with mortality, irrespective of treatment type (25). Similarly, in the case of nonagenarians undergoing surgery for odontoid fractures, the mortality rates are associated with preexisting comorbidities and are not related to the surgical approach (28). The results of the present study also support the notion that comorbidities are a major risk factor contributing to mortality.

Interestingly, according to our findings, the in-hospital and 90-day mortality rates were substantially lower at 5.0% compared to the rates described in the literature (9). Three more death were observed due to the follow up period due to acute heart failure, thus the overall mortality increased at 15.0%. Notwithstanding, also the overall mortality rates of the present study are still comparable lower than the ones demonstrated in previous studies (9). One potential explanation might be that our patients were monitored after surgery at the ICU or an intermediate care ward and were not directly transferred to the regular ward. Therefore, the closer attention the frail patients received at the ICU may have permitted an early diagnosis and better management of any potential complications.

4.2.2. Importance of ICU admission

According to our findings, the overall complication rates were relatively high at 50% with pneumonia being the most prevalent, followed by urinary tract infection, acute heart failure, and pleural effusion. Vaccaro et al. in their prospective study on older patients undergoing either surgery or conservative treatment for odontoid fractures reported a slight trend toward higher complication rates in the conservative group (30%) compared to that in the surgical group (36%) (30). Similar to our findings, pneumonia, respiratory problems, or dysphagia were quite frequently observed as adverse events post-surgery (30). Likewise, Charles et al. found an increased prevalence of complications with increasing age, and general medical complications such as respiratory and cardiac-related complications and delirium were most frequently noted irrespective of treatment type (29). One might argue that our complication rates were much higher than those reported by Vaccaro et al. and Charles et al. However, this disparity can be explained by the fact that our cohort constituted solely of patients aged 80 years and older; hence, our patients were more prone to experiencing adverse events attributable to their poor clinical profiles (CCI > 6). In agreement with our findings, Smith et al. found at least one major complication in 60% of the octogenarians undergoing surgery, with respiratory complications being the most prevalent (25). In the case of nonagenarians undergoing surgery for such fractures, the complication rate was

relatively high (5/15, 33%), with respiratory complications being the most frequent (28). Due to the post-operative admission to the ICU, we were able to immediately detect clinical worsening and swiftly initiate therapy, such as antibiotic treatment in the case of pneumonia, preventing potential development of devastating infectious complications such as septic events. In a large Australian study on long term survival outcomes after ICU admission, older patients (mean age ≥70 years) had better survival rates even 9 years after surgery if they were admitted to an ICU post-surgery (31). According to a recent review and meta-analysis, older patients and especially octogenarians benefit from the post-operative admission to ICU, which contributes to lower morbidity and mortality rates, especially in emergency settings such as after emergency surgery (32). Although evidence is conflicting regarding the benefits of ICU admission after surgery in younger patients, we feel that the close monitoring of such debilitated subset of patients resulted in the low mortality rates presented here. Therefore, we hypothesize that, in case of emergency surgery of octogenarians, ICU therapy may lead to better outcomes, especially with respect to long term survival rates.

In the present study, fusion was achieved in all patients and the neurological status also improved significantly. Herein, it should be emphasized that the five reported deaths were caused by pneumonic sepsis, whereas surgical complications were not observed. The overall mortality rate was 15% over a 4-year follow-up period, and the deaths were not related to the surgery (cause of death: acute heart failure and malignant stroke). Given the dearth of robust evidence, no definitive conclusion can be drawn regarding the optimal therapy for odontoid fractures, especially in octogenarians. The largest study (165 operative and 157 conservative management patients) on this topic was conducted by AOSpine North America. This study provided level III evidence and advocated that surgical management should be favored, as conservative therapy conferred a higher risk of mortality when adjusting for confounding factors such as age and comorbidities (33). Furthermore, Vaccaro et al. suggested that operative management of such fractures leads to better functional outcomes, but this did not reach statistical significance (30). Kuntz et al. in their retrospective review on older patients (>65 years) found that conservative management results in higher failure rates with morbidity and mortality rates that were comparable to those associated with surgical management (34). In another study based on claim data and including 3,847 octogenarians, the study group reported that patients undergoing surgery did not show a higher incidence of in-hospital mortality, though higher rates of complications were observed (35).

4.2.3. Surgical technique and intraoperative blood loss

The approach for the C1 lateral mass screws can be difficult, which is attributable to potential epidural bleeding from the venous plexus. This results in both a blockage in the surgeon's view and difficulty in drill placement. Compared with the surgical techniques described in literature, the modified C1 technique presents with an advantage: after performing a midline approach and subperiosteal exposure of bony structures, the intraoperative CT scan allows for heightened accuracy of navigation-assisted techniques. The intraoperative insertion of reference markers in the surgical field allows for a quick registration by touching clearly defined landmarks, which can be repeated in less than 1 min in case of insecurity or movement due to instability. Anatomical landmarks or prolonged surface matching are not needed. However, potential hindrances can occur depending on the patient's anatomy. For instance, in a prospective series, Yeom et al. described 51 Asian patients undergoing C1 fixation in a similar manner; however, anatomic considerations, such as a craniocaudal dimension of less than 4 mm of the C1 posterior arch, are most likely associated with cortical perforation (36). Furthermore, another difficulty of this technique is the presence of a smaller Atlas arch, a phenomenon usually seen in the Asian population (36). In addition, the presence of the ponticulus posticus, an ossification arch surrounding the dorsal course of the vertebral artery on the C1 posterior arch, endangers sagittal referencing from the superior boder of the posterior arch (37). Recognition of such anatomic variations via X-rays or CT imaging before surgery is pivotal in preventing tremendous complications during surgery. In the present study, no anatomical anomalies or variations were observed in preoperative imaging; thus, this technique could be applied without complications or damaging important structures such as the vertebral artery, the occipital nerve, as well as the C2 nerve root. Most importantly, bleeding from the epidural venous plexus can be avoided. By using an intraoperative navigation CT, we were able to track the patient's anatomy in real time, thereby reducing errors and enhancing clinical outcomes.

Although we used a modified technique for the insertion of C1 pedicle screws as described by Tan et al. (38), we observed higher intraoperative blood loss with a mean of approximately 700 ml, which is much higher than described in previous studies (28, 39). One potential explanation may be that over 50.0% of our cases were taking anticoagulant agents, which may have contributed to higher intraoperative blood loss. Although administration of the agents was stopped before surgery, most of surgeries were performed in the acute setting; thus, the medications presumably still had an effect, as confirmed by the PTT results. Even if patients received antidotes before surgery, we still observed substantial intraoperative bleeding. This phenomenon may be attributable to the fact that octogenarians, due to their pure baseline history with multiple prolonged comorbidities, may require discontinuation intervals since their renal function is substantially impaired (creatinine clearance of less than 30 ml/min) (33). Impaired renal function will lead to prolonged effects of anticoagulant agents despite discontinuation before surgery or the use of antidotes to counter their effect (34, 35). We surmise that these two factors are inciting events resulting in higher intraoperative bleeding, despite the modified surgical technique associated to minimize intraoperative blood loss. Thus, a meticulous study of this debilitating cohort should be conducted preoperatively to prepare spine surgeons to sufficiently deal with such intraoperative complications.

4.2.4. Conservative vs. surgical management

The optimal management of odontoid fractures type II, especially in octogenarians, remains a subject of debate. The union rates of type II odontoid fractures present the main factor for decision making and whether to initiate a conservative

or surgical treatment approach. Previous studies suggest that non-surgical management with a cervical collar or halo vest are associated with high non-union rates of approximately >40% (28, 36), while surgical management with posterior screw fixation increases the fusion rates to >80% (28, 36). Gembruch et al., in their retrospective analysis of 125 patients with a mean age of 85.7 years sustaining a type II odontoid fractures, reported union rates of 70.0% in the conservative group, while a fusion rate of almost 91.0% was achieved in patients undergoing C1/C2 posterior screw fixation (37). Of note, the in-hospital and 90-day mortality did not differ significantly between both groups, while an increase was observed after 90 days, attributable to cardiopulmonary diseases and not directly to surgery. Considering these points, we concluded that surgical management seems to produce more beneficial outcomes for the elderly due to the high fusion rates, when compared to conservatively treated patients (37). In another retrospective study on conservative treatment among 58 older patients with type II odontoid fractures, Smith et al. reported high a mortality rate of 14% after 90 days of conservative treatment, while 64.0% required surgery due to substantial development of non-union fractures. Importantly, another 22.0% of the cases underwent surgery due to secondary non-union (38). In conjunction with the abovementioned studies, our patients also underwent surgery due to initial spinal instability. The major difference between our study and the aforementioned ones (37, 38) is that our patients presented with an acute trauma that caused the type II odontoid fracture; most importantly, a non-union displacement >4 mm, posterior displacement, as well as fracture coagulation of more than 10° were present. Hence, surgery was the only efficient approach to prevent further instability or even spinal cord injury due to instability at the atlantoaxial junction.

4.2.5. Fusion rates after surgery or conservative management

In the current study, posterior screw fixation led to union rates of 100%. In concert with these findings, Issa et al., in their retrospective study on 15 nonagenarians who sustained acute odontoid type II fractures, reported fusion in all patients after C1-C2 pedicle screw fixation (28). Furthermore, Gembruch et al. also described higher fusion rates of 91.0% in patients undergoing surgery for type II odontoid fractures when compared to conservative management (37). Of note, the non-union rates after conservative management (over 30%) were high (37). Although, in accordance with our results and the aforementioned studies, surgical management seems to produce better outcomes for elderly patients, it should be noted that octogenarians are amenable to higher complications rates, especially after a surgical procedure, due to their poor baseline reserve. However, surgery seems to be inevitable when predisposing factors such as posterior displacement of the fracture, fracture distraction, lateral displacement on the posterior radiograph, and displacement greater than 4 mm (16) are present, as in the patients presented in the current study. It should be emphasized that posterior techniques are recommended in older patients since anterior screw fixation due to osteoporotic bones results in significantly lower fusion rates and is associated with higher morbidity and mortality rates (30). There is no clear consensus yet how to optimally treat such patients, but considering the results of the present study as well as of previous literature, posterior screw fixation unveils great rates of fusion which contributes to an improvement in the patient's quality of life.

We believe that our data suggest that the decision on whether elderly patients should undergo surgery for a Type-II dens fracture should not be based on age alone, but on the value of regaining or preserving their quality of life. We found that while these patients presented with increased risks of morbidity and mortality, their neurological condition improved after surgery and most importantly, fusion could be achieved. A future prospective randomized trial is required in which elderly patients are allocated to specific arms of operative or non-operative management and are stratified by age. Such a study will help clarify the optimal therapeutic intervention for elderly patients with Type-II dens fractures.

4.3. Limitations

The main strength of the current study is that it is the first study to examine such a large cohort of octogenarians undergoing surgery for odontoid fractures. However, there are some limitations to this study. Selection bias could not be ruled out because of the retrospective study design. Additionally, as the data originated from a high-volume center, potential performance bias should also be considered. A longer follow-up period may uncover other relevant information that was not captured in the current study. Functional outcomes could not be sufficiently reconstructed using patients' medical records; hence, they were omitted from this analysis. However, we believe that the impact of surgery could be adequately evaluated using the ASIA grading system. Our study employs a modified Goel-Harms technique, which, while rooted in the traditional approach, introduces specific variations. These modifications were consistently applied but may introduce outcomes slightly different from the conventional technique. However, the clinical significance of such nuances, given the foundational consistency with the original method, is expected to be minimal. Since patients suffered from an acute trauma and the admission to the hospital was less than 24 h after the initial trauma, a delay in the diagnosis was absent. All CT images showed the abovementioned parameters, as evaluated by experienced neuroradiologists and spine surgeons (KK, BI). Since octogenarians carry the high risk of perioperative morbidity and mortality, an interdisciplinary discussion was held with experienced anesthesiologists to evaluate the potential risks associated with surgery. However, since the enrolled patients suffered from unstable fractures which are unlikely to achieve a bony union by treatment by a halo vast and can presumably also result in progressive myelopathy and contribute to spinal cord injury due to instability at the atlantoaxial junction (16, 40, 41), a surgical procedure via posterior screw fixation was recommended, while a conservative treatment approach was considered as insufficient and associated with undue risks for

progression of spinal instability. Finally, a lack of a conservatively treated control group is a major limitation of this study.

5. Conclusion

With the global trend of increasing life expectancy due to accelerating improvements in the quality of healthcare worldwide, spine surgeons are frequently confronted with the management of odontoid fractures in the geriatric population. The complication and mortality rates of such fractures in octogenarians are relatively high. However, bone union and preservation of the neurological status are also major goals of the therapeutic strategies. Despite an often high comorbidity rate, we still recommend that surgery should be considered in patients over 80 years of age. A variety of approaches should be evaluated by healthcare professionals when treating elderly frail patients. Regardless, a clear discussion with the patient and relatives regarding the potential risk is unambiguously recommended.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

BI and PL performed material preparation and data collection and analysis. PL wrote the first draft of the manuscript. GG, KK, AU, and BI commented on previous versions of the manuscript. All authors contributed to the study conception and design, read, and approved the final manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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