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Metals accumulation affects bone and muscle in osteoporotic patients: A pilot study

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ABSTRACT

Osteoporosis is the most common bone disease, characterized by decreased bone mineral density (BMD) and often associated to decreased muscle mass and function. Metal exposure plays a role in the pathophysiology of osteoporosis and affects also muscle quality. The aim of this study was to assess the association between metal levels in bone and muscle samples and the degeneration of these tissues. A total of 58 subjects (30 male and 28 female) was enrolled and classified in osteoporotic (OP, n = 8), osteopenic (Ope, n = 30) and healthy (CTR, n = 20) subjects, according to BMD measures. Femoral head bone samples and *vastus lateralis* muscle samples were collected during hip arthroplasty surgeries. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis showed increased levels of Al, Cd and Pb in OP and Ope bone tissue compared to CTR subjects ($p = 0.04$, $p = 0.005$ and $p = 0.01$, respectively). Whereas, increased levels of Co, Cd and Pb were measured in OP and Ope muscle tissues, compared to CTRs ($p < 0.001$, $p = 0.02$ and $p = 0.01$, respectively). In addition, Al, Cd and Pb levels in bone and Cd and Co levels in muscle were negatively correlated with BMD. A negative association among Co, Cd, Cr and Hg levels and muscle fibers diameter was also observed in muscle tissues. This study assessed that metal exposure can affects bone and muscle tissue quality and may contribute to the onset and progression of musculoskeletal diseases such as osteoporosis. Therefore, it is important to implement metal exposure assessment and their impact on disease development, in order to manage and prevent metal accumulation effects on bone and muscle quality.

1. Introduction

Osteoporosis represents the most common disease of the musculoskeletal system, which just in Italy affects approximately 5 milion people (Marcellusi et al., 2020). It is characterized by progressive decreased of bone mineral density (BMD) and microarchitectural changes, which lead to a deterioration of bone tissue, and resulting in an increased bone fragility and susceptibility to fractures (Cerocchi et al., 2013; Piccirilli et al., 2022). The decrease in BMD below normal reference values, yet not low enough to meet the diagnostic criteria for osteoporosis, are described as osteopenia. In fact, osteoporosis is defined by a *t*-score value of less than −2.5, while a *t*-score value between −1 and −2.5 defines a condition of osteopenia (Varacallo et al., 2023).

The pathogenesis of osteoporosis is represented by a complex of interactions between local and systemic regulatory factors of bone cells function, that results in an imbalance of the bone remodeling process to which bone tissue is constantly subjected (Drake et al., 2015; Florencio-Silva et al., 2015). During motion, the mechanical stimulus exerted by the muscle results in an adaptive response by the bone, which involves an improvement in both the mass and strength of the bone

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tissue itself. The bone appears to be intimately connected with the muscle, not only anatomically, but also biomechanically and biochemically, together constituting the functional bone-muscle unit (Tarantino et al., 2022). Therefore, pathophysiological changes that occur in bone are reflected in changes of muscle tissue, and *vice versa* (Yu et al., 2023). In this perspective, muscle and bone are not only regulated by several factors, but also regulate each other (Yu et al., 2023). The decrease in bone quality is often associated to a progressive decrease in muscle mass and strength, a condition known as sarcopenia, which can increase the risk of falls and fractures (Tarantino et al., 2021). The number of people worldwide that will develop sarcopenia in 2040 is estimated to be more than 200 million. It is noteworthy that an increase in the prevalence of osteoporosis of 78.1% was observed in the elderly sarcopenic subjects, compared to 47.6% in non-sarcopenic subjects (Cruz-Jentoft et al., 2010; Kirk et al., 2020a).

There are common risk factors related to the onset and progression of bone-muscle diseases; among them, metals exposure could play a relevant role (Clynes et al., 2021; Tarantino et al., 2022). Metals are environmental pollutants found worldwide that can bioaccumulate in the human body following exposure by inhalation, ingestion or epidermal contact. Some metals are necessary for maintaining physiological functions, such as copper (Cu), molybdenum (Mo), selenium (Se), and zinc (Zn). On the other hand, exposure to metals such as arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb), may be associated to the occurrence of adverse health effects (Ajibo et al., 2023). Moreover, environmental exposure to metals appears to be related to a deregulated gene expression pattern, leading to increased susceptibility to the development of several diseases, including osteoporosis (Lim et al., 2016; Visconti et al., 2023). Metals exposure appears to negatively affect bone mass, since bone could be the primary target for accumulation; in any case, the role of bone exposure to toxic metals in the pathogenesis of osteoporosis has yet to be clarified (Sadeghi et al., 2014). The biological half-life of metals in bone tissue lasts up to 30 years, thus this tissue is a potentially useful biomarker of cumulative exposure for several metals. Noteworthy, up to 90% of the body burden of metals is stored in bone (Hasan et al., 2020; Zaichick et al., 2011). Such metals appeared to deposit in bone tissues and negatively affect bone health, due to their ability to compete with calcium (Ca) in bone matrix, alter the normal bone cells development and/or damage second organ as kidneys, affecting the Ca absorption-excretion balance (Rodríguez and Mandalunis, 2018; Scimeca et al., 2017). Lead represents the toxic metal most associated with the onset of bone diseases, as this could accumulates in the bone early in fetal development, causing growth retardation through inhibition of endochondral ossification process (Khalil et al., 2008). Lead appears to alter bone turnover by promoting bone resorption, thus leading to a decrease in the rate of mineralization and BMD levels. The alteration of bone turnover is due to the cytotoxic effect of Pb on osteoclasts, osteoblasts and chondrocytes. Lead slows down the maturation of chondrocytes, resulting in an impairment of the entire remodeling process. This alteration is also due to the deleterious effect on some bone matrix proteins including osteopontin, osteocalcin and collagen (Dermience et al., 2015). Recently, *in vitro* studies also showed how Pb, together with Cd, exerts a cytotoxic effect on osteoblasts, leading to increased levels of cellular oxidative stress and concomitant impairment of antioxidant defense systems, through reduced activity of superoxide dismutase (SOD), catalase (CAT) and cellular glutathione levels (GSH) (Al-Ghafari et al., 2019). In agreement, further studies reported that Cd blood levels are correlated with poor bone quality, in terms of decreased trabecular bone score (TBS), and a high prevalence of osteoporosis (Huang et al., 2023a; Huang et al., 2023b). Accordingly, it has been observed that Cd and Pb exposure was associated with an increased risk of osteopenia or osteoporosis, but also other metals, as Al, Co, Hg, Mn, could play a role in the pathogenesis (He et al., 2023; Huang et al., 2023a; Lim et al., 2016; Scimeca et al., 2017; Visconti et al., 2023). Moreover, Cd together with chromium (Cr) is able to exert negative effects on osteoblasts proliferation and activity, leading to decreased levels of alkaline phosphatase and Ca deposition (Soudani et al., 2011). Conversely, cobalt (Co) appears to be associated with modulation of bone cells metabolism, thus affecting bone resorption and formation processes, but also with increased levels of cellular oxidative stress (Dermience et al., 2015; Fleury et al., 2006). Recently, Scimeca et al. observed the prevalence of Pb, Cd, and Cr in the bone tissue of osteoporotic patients by scanning electron microscopy (SEM) investigations. Moreover, the presence of these metals correlated with the expression of sclerostin in the same tissues, suggesting a possible link between the accumulation of toxic metals and altered bone metabolism (Scimeca et al., 2017).

However, considering the bone-muscle unit, the accumulation of metals may be related to the degeneration of both tissues. Recent studies have shown that exposure to Cd, Co, Hg, Mn, and Pb affects muscle weakness, potentially being associated with increased risk of sarcopenia (Huang et al., 2023b). Nevertheless, the actual associations between metal exposure and the pathogenesis of osteopenia and osteoporosis were still unclear and require further investigation. Moreover, the metal effects on muscle quality were studied to a limited extent.

In the present study aluminium (Al), As, Cd, Co, Cr, Cu, Mn, nichel (Ni), Hg, Pb, Se, stronzium (Sr) and Zn in bone and muscle tissues of osteoporotic (OP), osteopenic (Ope) and healthy subjects (CTR) were quantified. The association between the concentration of these metals and quality of both tissues was also studied. Moreover, given the intimate connection between bone and muscle tissues, we hypothesized that metal levels in muscle could affect tissue quality and be implicated in osteoporotic diseases. Therefore, the aim of the present study is to expand the knowledge of metal exposure as risk factor for BMD decrement and muscle atrophy that typically characterizes osteoporotic pathology.

2. Materials and methods

2.1. Study population

A total of 58 subjects (30 males and 28 females) were enrolled for this study, from September 2021 to June 2022. Subjects underwent surgery in the Orthopaedics and Traumatology Unit of Tor Vergata Polyclinic (Rome, Italy), for fragility fractures following low-energy trauma or high-energy fractures. Bone tissue samples (n = 49) taken from the excised femoral head and muscle tissue samples (n = 40) taken from the *vastus lateralis* muscle were collected during hip arthroplasty surgeries. After surgical resection, tissue samples were transported to the laboratory of the Hygiene Section of the University of Rome "Tor Vergata" and stored at −80 °C until spectrometry analysis was performed. It was not possible for each patient to collect both bone and muscle tissue samples due to surgery procedures. Individuals affected by malignancies, endocrine disorders affecting bone and mineral metabolism, autoimmune diseases, and bone disorders other than primary osteoporosis were excluded from the study, as well as those who underwent long-term therapy with drugs interfering with bone metabolism, sex hormone replacement therapy, antifracture and/or osteoanabolic therapies. The study was approved by the Ethical Board of "Policlinico Tor Vergata" (approval reference number #17/21). Informed consent was obtained from all the participants and all experimental procedures were carried out according to the code of ethics of the World Medical Association (Declaration of Helsinki).

2.2. Clinical and biochemical parameters

Information on participants' characteristics, including age (years), gender, weight and height to calculate body mass index (BMI), were obtained from medical records. The diagnosis of osteoporosis was based on dual-energy X-ray absorptiometry (DXA) performed before surgery, and the measure of BMD was carried out in each subject with a Lunar DXA apparatus (GE Healthcare, Madison, WI, USA). Lumbar spine

Table 1
Characteristics of osteoporotic (OP), osteopenic (Ope) and control (CTR) subjects (n = 58). Data are presented as median (mean ± sd).

Parmeter	OP (n = 8)	Ope (n = 30)	CTR (n = 20)	[a]Kruskal-Wallis test	[b]post-hoc pairwise comparison test		
					OP-CTR	Ope-CTR	OP-Ope
Female sex (%)	63	70	10	**< 0.001**	**< 0.001**	**< 0.001**	**0.69**
Age (years)	72.0 (71.5 ± 9.7)	72.5 (71.3 ± 9.8)	60.5 (57.2 ± 18)	**0.01**	**0.05**	**0.004**	0.94
BMI (kg/cm^2)	24.9 (24.6 ± 2.1)	25.1 (25.8 ± 4.3)	29.0 (29.2 ± 4.2)	**0.01**	**0.01**	**0.004**	0.65
BMD total femur (g/cm^2)	0.76 (0.78 ± 0.08)	0.84 (0.86 ± 0.08)	1.08 (1.12 ± 0.13)	**< 0.001**	**< 0.001**	**< 0.001**	0.11
t-score total femur	−1.95 (−1.84 ± 0.59)	−1.40 (−1.25 ± 0.68)	0.50 (0.85 ± 1.1)	**< 0.001**	**< 0.001**	**< 0.001**	0.09
BMD femoral neck (g/cm^2)	0.71 (0.71 ± 0.05)	0.83 (0.83 ± 0.09)	0.99 (1.01 ± 0.07)	**< 0.001**	**< 0.001**	**< 0.001**	**0.01**
t-score femoral neck	−2.45 (−2.43 ± 0.43)	−1.50 (−1.48 ± 0.68)	−0.40 (−0.22 ± 0.52)	**< 0.001**	**< 0.001**	**< 0.001**	**0.01**
BMD lumbar vertebrae L1-L4 (g/cm^2)	0.93 (0.99 ± 0.25)	1.09 (1.10 ± 0.12)	1.25 (1.38 ± 0.29)	**< 0.001**	**< 0.001**	**< 0.001**	0.23
t-score lumbar vertebrae L1-L4	−1.80 (−1.34 ± 2.1)	−0.80 (−0.69 ± 1.01)	0.55 (1.69 ± 2.4)	**< 0.001**	**< 0.001**	**< 0.001**	0.53
[c]Muscle fibers diameter (μm)	71.9 (70.3 ± 3.3)	72.4 (69.8 ± 10)	80.3 (83.9 ± 14)	**0.04**	**0.05**	**0.02**	0.77
Ca (mg/dL)	8.25 (8.19 ± 0.27)	8.40 (8.45 ± 0.46)	8.40 (8.34 ± 0.43)	0.25	ns	ns	ns
25-(OH)-VitD (ng/mL)	18.7 (18.2 ± 7.2)	19.1 (18.0 ± 9.3)	20.4 (21.8 ± 7.8)	0.42	ns	ns	ns
PTH (pg/mL)	92.3 (95.4 ± 28)	81.4 (82.8 ± 29)	70.6 (74.4 ± 21)	0.16	ns	ns	ns

[a]p-values obtained by Kruskal-Wallis test ($p < 0.05$).
[b]p-value obtained by post-hoc pairwise comparison test ($p < 0.05$).
[c]muscle fibers diameters were obtained for 4 OP, 24 Ope and 12 CTR subjects.
BMI, body mass index; BMD, bone mineral density; Ca, calcium; 25-(OH)-Vit D, 25-hydroxyvitamin D; PTH, parathyroid hormone; ns, non-significant.

(L1–L4) and femoral (neck and total) scans were performed according to the manufacturer's recommendation (Celi et al., 2013). The unit of measurement is represented by standard deviation (sd) from the mean BMD peak (*t*-score), with a coefficient of variation of 0.7%, on the uninjured limb. According to the World Health Organization (WHO), the subjects were divided in three groups according to BMD measured as *t*-score: *t*-score < −2.5, osteoporotic (OP) group; *t*-score between −2.5 and −1.0, osteopenic (Ope) group; and *t*-score > −1, control (CTR) group, i.e. the general population that does not report symptoms of osteoporosis. Histomorphometric analysis of muscle tissue biopsies was performed; specifically, muscle fibers diameter was measured as an indicative parameter of muscle quality status (see supplementary materials). Finally, Ca, parathyroid hormone (PTH), and 25-hydroxyvitamin D [25-(OH)-VitD] levels were measured in fasting venous blood samples.

2.3. Analysis of metal levels

The iCAP-RQ Inductively Coupled Plasma Mass Spectrometry (ICP-MS), with the collision Q-Cell and the Kinetic Energy Discrimination technology (KED), was used to detect in bone and muscle tissue samples the following metals: Al, As, Cd, Co, Cr, Cu, Mn, Ni, Hg, Pb, Se, Sr and Zn

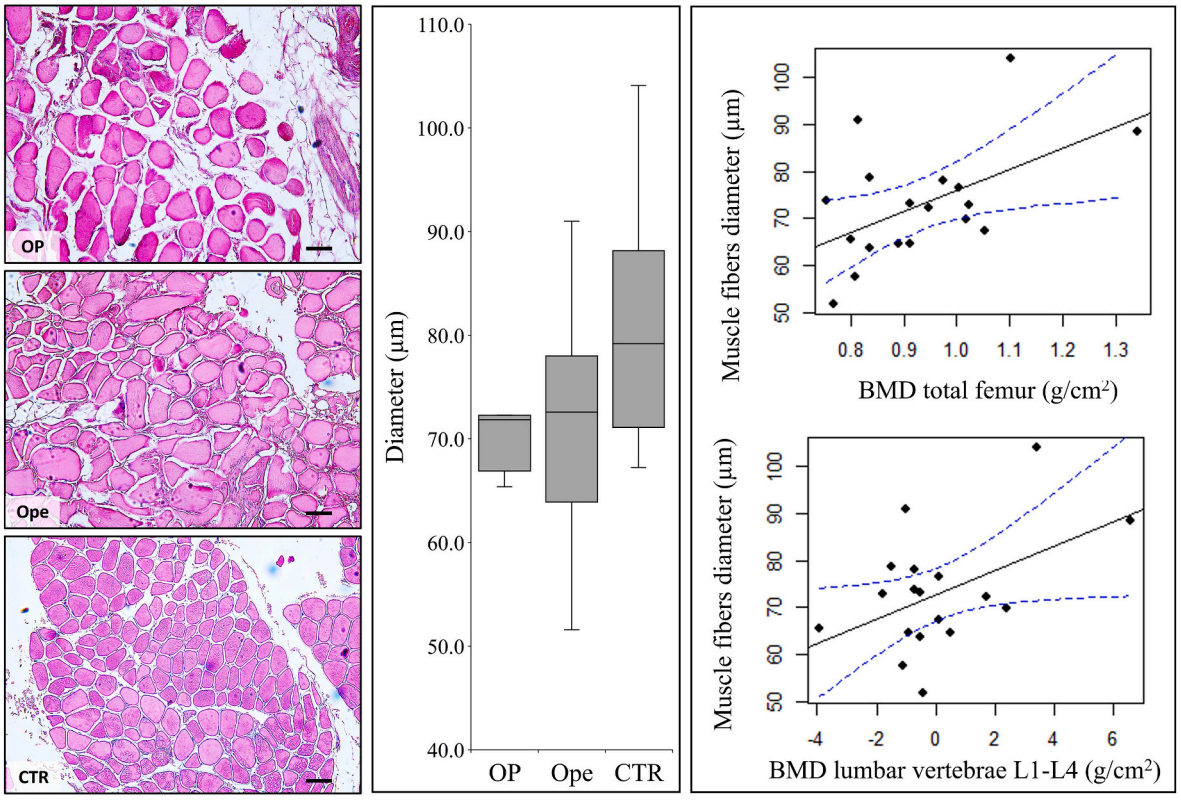

Fig. 1. Association between muscle and bone quality. Muscle fibers of OP, Ope patients and CTR subjects (magnification 10x, scale bar: 100 μm). The diameter of muscle fibers was lower in OPs and Ope than in the CTR subjects group ($p = 0.04$). Linear regression graphs show a significant positive association between muscle fibers diameter and BMD of total femur and lumbar vertebrae L1-L4.

Table 2
Metal levels (mg/kg) in bone tissue of osteoporotic (OP), osteopenic (Ope) and control (CTR) subjects (n = 48). Data are presented as median (mean ± sd).

Metal (mg/kg)	OP (n = 7)	Ope (n = 27)	CTR (n = 14)	[a]Kruskal-Wallis test	[b]post-hoc pairwise comparison test		
					OP-CTR	Ope-CTR	OP-Ope
Al	10.0 (15.4 ± 8.9)	7.30 (8.89 ± 7.0)	5.82 (5.93 ± 3.2)	**0.04**	**0.01**	0.29	**0.05**
As	0.0031 (0.0040 ± 0.002)	0.0049 (0.0063 ± 0.004)	0.0042 (0.0044 ± 0.002)	0.18	ns	ns	ns
Cd	0.018 (0.019 ± 0.005)	0.016 (0.018 ± 0.009)	0.0080 (0.010 ± 0.006)	**0.005**	**0.01**	**0.003**	0.51
Co	0.011 (0.010 ± 0.004)	0.0074 (0.0014 ± 0.01)	0.011 (0.012 ± 0.007)	0.51	ns	ns	ns
Cr	3.42 (2.97 ± 1.6)	1.13 (1.87 ± 1.7)	1.32 (1.70 ± 1.3)	0.19	ns	ns	ns
Cu	0.31 (0.33 ± 0.2)	0.31 (0.35 ± 0.3)	0.18 (0.34 ± 0.3)	0.86	ns	ns	ns
Hg	0.0021 (0.0036 ± 0.003)	0.0029 (0.0041 ± 0.004)	0.0032 (0.0041 ± 0.003)	0.75	ns	ns	ns
Mn	0.20 (0.18 ± 0.1)	0.13 (0.34 ± 0.4)	0.18 (0.24 ± 0.2)	0.79	ns	ns	ns
Ni	0.04 (0.11 ± 0.2)	0.06 (0.11 ± 0.1)	0.08 (0.10 ± 0.1)	0.63	ns	ns	ns
Pb	1.67 (1.61 ± 0.5)	1.06 (1.11 ± 0.6)	0.57 (0.85 ± 0.6)	**0.01**	**0.002**	0.10	**0.04**
Se	0.11 (0.12 ± 0.03)	0.13 (0.15 ± 0.1)	0.16 (0.19 ± 0.1)	0.40	ns	ns	ns
Sr	30.3 (29.1 ± 6.6)	30.9 (35.5 ± 15)	28.9 (30.5 ± 14)	0.61	ns	ns	ns
Zn	80.5 (76.3 ± 12)	71.2 (77.1 ± 18)	92.4 (89.7 ± 29)	0.30	ns	ns	ns

[a]p-values obtained by Kruskal-Wallis test ($p < 0.05$).
[b]p-value obtained by post-hoc pairwise comparison test ($p < 0.05$).
ns, non-significant.

(Table S1). The qualitative performances of ICP-MS were reported in Tables S2 and S3 for bone and muscle samples, respectively (see supplementary materials). The limits of detection (LoDs) in bone tissues were the following (mg/kg): 0.04, Al; 0.001, As; 0.001, Cd; 0.004, Co; 0.008, Cr; 0.008, Cu; 0.001, Hg; 0.006, Mn; 0.006, Ni; 0.02, Pb; 0.01, Se; 0.04, Sr; 0.06, Zn. The LoDs in muscle tissues were the following (mg/kg): 0.08, Al; 0.004, As; 0.006, Cd; 0.007, Co; 0.02, Cr; 0.04, Cu; 0.003, Hg; 0.009, Mn; 0.02, Ni; 0.02, Pb; 0.02, Se; 0.03, Sr; 0.18, Zn. The mean recovery rates ranged between 89.2% for Zn and 112.2% for Cr in bone tissues and 91.3% for Ni and 105.5% for Pb in muscle tissues; whereas the precision was lower than 6.5% in bones and 4.2% in muscle. The method accuracy for bone tissue evaluated on CRM 1486 (NIST) were from 85.5% to 116.4%; while for muscle tissue evaluated on CRM BB184 (IRMM) were from 78.9% to 112.3 %.

2.4. Statistical analysis

Standard statistical procedures were used to calculate median, mean

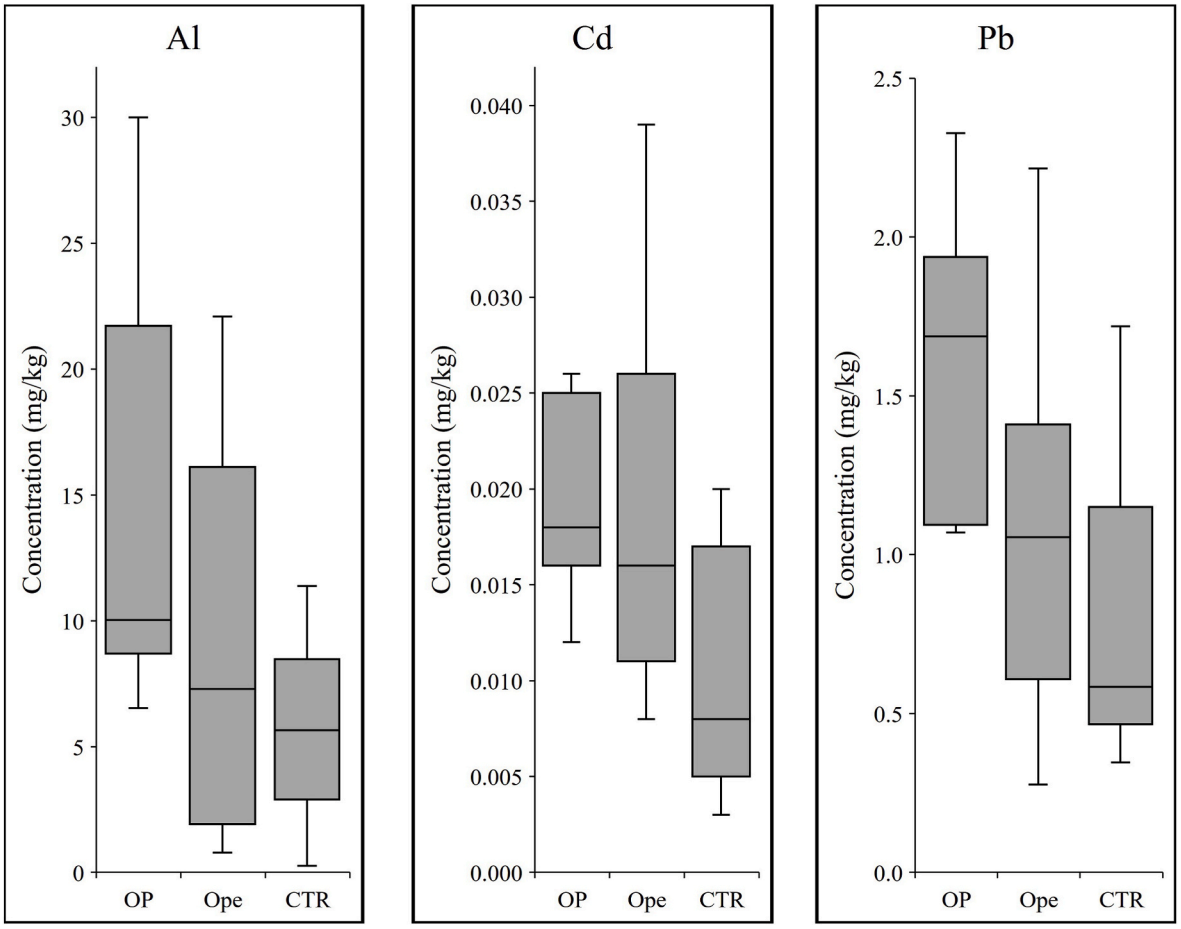

Fig. 2. Comparison of Al, Cd and Pb levels (mg/kg) in bone tissues of osteoporotic (OP), osteopenic (Ope) patients and healthy (CTR) subjects ($p = 0.04$, $p = 0.005$ and $p = 0.01$, respectively).

Table 3
Metal levels (mg/kg) in muscle tissue of osteoporotic (OP), osteopenic (Ope) and control (CTR) subjects (n = 40). Data are presented as median (mean ± SD).

Metal (mg/kg)	OP (n = 7)	Ope (n = 27)	CTR (n = 14)	[a]Kruskal-Wallis test	[b]post-hoc pairwise comparison test		
					OP-CTR	Ope-CTR	OP-Ope
Al	4.94 (4.76 ± 1.2)	4.77 (4.99 ± 3.1)	3.26 (3.56 ± 2.1)	0.32	ns	ns	ns
As	0.017 (0.025 ± 0.02)	0.0081 (0.018 ± 0.03)	0.010 (0.014 ± 0.01)	0.33	ns	ns	ns
Cd	0.61 (0.60 ± 0.3)	0.20 (0.17 ± 0.1)	0.040 (0.064 ± 0.09)	**< 0.001**	**< 0.001**	**0.01**	**0.01**
Co	0.25 (0.27 ± 0.2)	0.032 (0.10 ± 0.2)	0.012 (0.052 ± 0.1)	**0.02**	**0.004**	0.12	**0.04**
Cr	1.88 (1.96 ± 0.5)	0.96 (1.18 ± 0.7)	0.82 (0.99 ± 0.7)	0.08	ns	ns	ns
Cu	2.99 (2.85 ± 0.8)	2.48 (2.35 ± 0.9)	2.33 (2.32 ± 1.0)	0.40	ns	ns	ns
Hg	0.035 (0.039 ± 0.01)	0.035 (0.042 ± 0.03)	0.025 (0.033 ± 0.03)	0.47	ns	ns	ns
Mn	0.39 (0.50 ± 0.3)	0.40 (0.43 ± 0.3)	0.31 (0.33 ± 0.2)	0.50	ns	ns	ns
Ni	0.30 (0.37 ± 0.3)	0.20 (0.26 ± 0.2)	0.11 (0.18 ± 0.2)	0.37	ns	ns	ns
Pb	0.88 0.82 ± 0.4)	0.15 (0.18 ± 0.2)	0.11 (0.12 ± 0.08)	**0.01**	**0.002**	0.48	**0.004**
Se	0.58 (0.58 ± 0.2)	0.58 (0.53 ± 0.2)	0.48 (0.46 ± 0.2)	0.29	ns	ns	ns
Sr	0.83 (0.86 ± 0.6)	2.49 (3.44 ± 3)	1.86 (2.35 ± 2)	0.10	ns	ns	ns
Zn	141.6 (144.9 ± 55)	158.3 (152.0 ± 69)	117.4 (128.0 ± 76)	0.66	ns	ns	ns

[a]p-values obtained by Kruskal-Wallis test ($p < 0.05$).
[b]p-value obtained by post-hoc pairwise comparison test ($p < 0.05$).
ns, non-significant.

and relative sd of the variables of the three independent groups. The differences among OP, Ope and CTR groups were investigated by non-parametric Kruskal-Wallis test, after checking the non-normally distribution. A post-hoc pairwise comparison test was carried out to identify the specific group values displaying statistical differences. The box plots were used to compare the different distributions of data among the three groups. Spearman's rank test was applied to assess the association among clinical and biochemical variables and metals level. Significant associations were showed as linear regression graphs with 95% confidence intervals. The presence of statistical differences was evaluated as a significance level of p-value less than 0.05 ($p < 0.05$). Data were analyzed with SPSS 25.0 (SPSS Inc., Chicago, IL, USA) and R (4.0.4 revised) statistic software.

3. Results

3.1. Clinical and biochemical characteristics

The study was performed on 58 subjects classified in OP (n = 8), Ope (n = 30) and CTR (n = 20) according to BMD measures; the clinical and biochemical characteristics of patients are shown in Table 1.

OP and Ope groups showed a higher female percentage than the CTR group ($p < 0.001$). Whereas age did not differ between OP and Ope groups (median, 72.0 and 72.5 years, respectively), it was significantly lower in the CTRs (60.5 years) compared with the other groups ($p = 0.01$). Similarly, BMI values showed difference ($p = 0.01$) between OP and Ope groups (median, 24.9 and 25.1 kg/cm^2, respectively) and the CTR group (median, 29.0 kg/cm^2). BMD and relative *t*-score of total femur, femoral neck and lumbar vertebrae L1-L4 showed significant difference among OP, Ope and CTR subjects ($p < 0.001$). The difference in BMD of the femoral neck was found statistically significant between OP-CTR ($p < 0.001$), Ope-CTR ($p < 0.001$) and OP-CTR ($p = 0.01$) by post-hoc test, otherwise BMD of total femur and lumbar vertebrae showed difference between disease groups and CTR.

Muscle fibers diameter was significantly smaller in the OP and Ope patients (median, 71.9 and 72.4 μm, respectively) compared to CTR subjects (median, 80.3 μm). In addition, positive association between muscle fibers diameter and BMD of total femur (0.52, $p = 0.02$) and BMD of lumbar vertebrae L1-L4 (0.48, $p = 0.04$) was detected. The linear regression graphs show the relationship between muscle fibers diameter and BMD (Fig. 1).

Otherwise, level of Ca, 25-(OH)-VitD and PTH showed no significant difference among OP, Ope and CTR subjects (Table 1).

3.2. Metal levels in bone tissues

Metal levels measured in bone tissue samples (n = 48) of OP, Ope and CTR subjects were reported in Table 2. Aluminium level was significantly different ($p = 0.04$) in bone tissues of OP (median, 10.0 mg/kg), Ope (median, 7.30 mg/kg) and CTR subjects (median, 5.82 mg/kg). Moreover, Cd level resulted comparable in OP and Ope groups (median, 0.018 and 0.016 mg/kg for Cd) and significantly higher ($p = 0.005$) than CTRs (median, 0.0080 mg/kg for Cd). Lead level resulted higher ($p = 0.01$) in OP group (1.67 mg/kg) compared to Ope (1.06 mg/kg) and CTRs (0.57 mg/kg). Pairwise comparison suggested that only the comparisons between OP-CTR and OP-Ope for Al and Pb were significant ($p \leq 0.05$). For Cd, the post-hoc test showed a significant difference between OP-CTR and Ope-CTRgroups ($p \leq 0.01$), but not between OP and Ope. Aluminium, Cd and Pb levels in bone tissue of three groups were reported in Fig. 2.

3.3. Metal levels in muscle tissues

Metal levels measured in muscle tissue samples (n = 40) of OP, Ope and CTR subjects were reported in Table 3. Cadmium level was significantly different among groups ($p < 0.001$) with a median of 0.61 mg/kg in OP, 0.20 mg/kg in Ope and 0.04 mg/kg in CTR muscle tissues. Pairwise comparison confirmed the statistic differences among the three groups with a $p < 0.001$ for OP-CTR, $p < 0.01$ for Ope-CTR and OP-Ope. Higher levels of Co and Pb were observed in OP groups (0.25 mg/kg and 0.88 mg/kg, respectively) respect to Ope (0.032 mg/kg and 0.15 mg/kg) and CTR (0.012 mg/kg and 0.11 mg/kg) groups ($p < 0.05$). This result was confirmed by the comparison between OP and CTR ($p < 0.005$) or Ope ($p < 0.05$) group, whereas Ope-CTR was reported as not statistically significant. Cadmium, Co and Pb levels in muscle tissue of three groups were reported in Fig. 3.

3.4. Association between bone metal accumulation and characteristics of subjects

Association of metal accumulation and bone tissues clinical parameter, such as BMD and *t*-score was investigated (Table 4). Negative association was observed between Al level in bone tissue and BMI (−0.39), BMD total femur (−0.36), *t*-score total femur (−0.33) and BMD lumbar vertebrae L1-L4 (−0.30). Cadmium level negatively correlated with BMD total femur and BMD femoral neck (−0.49 and −0.41, respectively) and relative *t*-score (−0.47 and −0.40, respectively). Also, Pb level showed negative association with BMD total femur and femoral neck (−0.33). In addition, metals bioaccumulation in bone did not show a association with the muscle fibers diameter. Finally, a positive

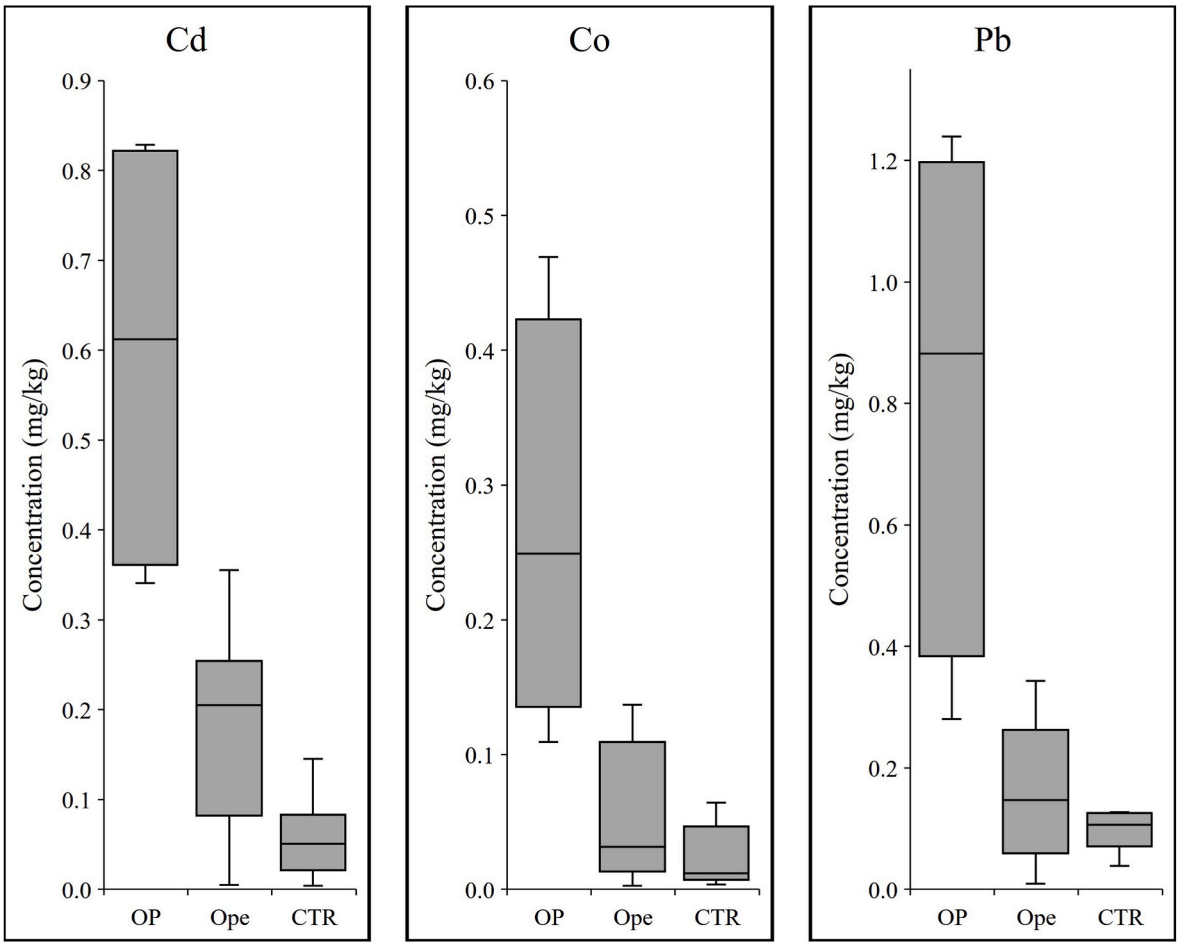

Fig. 3. Comparison of Cd, Co and Pb levels (mg/kg) in muscle tissues of osteoporotic (OP), osteopenic (Ope) patients and healthy (CTR) subjects ($p < 0.001$, $p = 0.02$ and $p = 0.01$, respectively).

association between As and age was detected (0.42). The significant associations are shown as linear regression graphs, to show the association among Al, Cd and Pb levels in bone tissue and BMD and *t*-score parameters (Fig. 4).

3.5. Association between muscle metal accumulation and characteristics of subjects

Association of metal accumulation in muscle tissue, bone clinical parameters and muscle fibers diameters was investigated (Table 5). Cadmium level negatively correlated with BMD total femur and BMD femoral neck (−0.47 and −0.42, respectively) and relative *t*-score (−0.50 and −0.42 respectively); in addition, Cd level was negatively correlated with BMI (−0.36). Conversely, a positive association between Cd level and age was observed (0.47). Negative association was detected among Co level and BMD total femur (−0.34), relative *t*-score (−0.43) and *t*-score femoral neck (−0.39). Cobalt level resulted positively correlated with age (0.48). Positive association between As level and BMD lumbar vertebrae L1-L4 was detected (0.39). In addition, Mn level and age resulted positively correlated (0.32). Regarding muscle quality, a negative association was observed among fibers diameter and Cd (−0.44), Co (−0.46), Cr (−0.53) and Hg (−0.48) levels. The significant associations are shown as linear regression graphs, to show the association among Cd, Co, Cr, Hg and Mn levels in muscle tissue, and BMD, *t*-score and muscle fibers diameter parameters (Fig. 5).

4. Discussion

Osteoporosis represents one of the most common age-related diseases of the musculoskeletal system, characterized by the decrease of BMD, which is reflected in a weakness of the bone tissue and an increase in fragility fractures (Cerocchi et al., 2013; Piccirilli et al., 2022). Gender, age and BMI were well-documented risk factors for osteoporosis. In our study, women were more likely to show a reduction in BMD than men, with a prevalence in both OP (63%) and Ope (70%) group. Similarly, De Martinis and colleagues showed a higher prevalence of osteoporosis among women (51.27%) compared to men (27.91%) on more than 2000 subjects (De Martinis et al., 2021). This was normally due to the lack of estrogens in postmenopausal age, leading to earlier bone loss than men (Ji and Yu, 2015). BMI values increased in OP and Ope groups respect to CTRs, meaning that it affects BMD. This result agreed with a previous study for which the optimal value of BMD to preserve and minimize the risk of osteoporosis was 26.9 kg/m^2 (Skrzek et al., 2014).

The bone and muscle mass loss often occurs together in older patients due to intimate anatomical, biomechanical, and biochemical connection between these tissues (Kirk et al., 2020b; Tarantino et al., 2022). In this study, histomorphometric analysis showed that OP and Ope groups were characterized by smaller muscle fibers diameter than healthy subjects, indicating the association with the severity of bone mass reduction. The diameter values in OP group (median, 71.9 μm) showed a lower degree of atrophy compared to the OP group measured by Terracciano et al. in which muscle fibers diameter ranged from 26.5 to 42.8 μm with a max of 36.9% of atrophic fibers (< 30 μm) (Terracciano et al., 2013). Moreover,

Table 4
Association between level of metals in bone tissue samples and subject characteristics.

Metal	Age	BMI	BMD total femur	*t*-score total femur	BMD femoral neck	*t*-score femoral neck	BMD lumbar vertebrae L1-L4	*t*-score lumbar vertebrae L1-L4	Muscle fibers diameter
Al	0.05 (p = 0.74)	**−0.39 (p = 0.007)**	**−0.36 (p = 0.007)**	**−0.33 (p = 0.02)**	−0.10 (p = 0.12)	−0.12 (p = 0.44)	**−0.30 (p = 0.03)**	−0.21 (p = 0.15)	−0.15 (p = 0.51)
As	**0.42 (p = 0.003)**	−0.13 (p = 0.339)	−0.001 (p = 0.39)	−0.01 (p = 0.96)	−0.04 (p = 0.79)	−0.03 (p = 0.84)	0.09 (p = 0.55)	0.04 (p = 0.80)	−0.12 (p = 0.61)
Cd	−0.10 (p = 0.50)	−0.09 (p = 0.55)	**−0.49 (p < 0.001)**	**−0.47 (p = 0.001)**	**−0.42 (p = 0.004)**	**−0.40 (p = 0.005)**	−0.21 (p = 0.17)	−0.17 (p = 0.24)	−0.07 (p = 0.75)
Co	0.04 (p = 0.78)	0.13 (p = 0.38)	0.24 (p = 0.11)	0.28 (p = 005)	0.13 (p = 0.39)	0.18 (p = 0.22)	0.06 (p = 0.71)	0.06 (p = 0.71)	0.40 (p = 0.07)
Cr	0.20 (p = 0.57)	−0.86 (p = 0.57)	0.07 (p = 0.64)	0.01 (p = 0.96)	0.08 (p = 0.59)	0.01 (p = 0.92)	−0.04 (p = 0.77)	0.01 (p = 0.97)	0.18 (p = 0.43)
Cu	−0.02 (p = 0.90	0.07 (p = 0.70)	−0.09 (p = 0.56)	0.02 (p = 0.89)	−0.16 (p = 0.30)	−0.06 (p = 0.71	−0.03 (p = 0.85)	−0.004 (0.98)	0.23 (p = 0.31)
Hg	0.12 (p = 0.42)	−0.14 (p = 0.34)	0.11 (p = 0.49)	0.10 (p = 0.51)	0.03 (p = 0.85)	−0.005 (p = 0.98)	0.10 (p = 0.53)	0.15 (p = 0.32)	0.17 (p = 0.46)
Mn	0.09 (p = 0.57)	0.12 (p = 0.43)	0.18 (p = 0.25)	0.22 (p = 0.14)	0.13 (p = 0.40)	0.18 (p = 0.22)	0.03 (p = 0.83)	0.03 (p = 0.86)	0.20 (p = 0.36)
Ni	0.16 (p = 0.29)	0.06 (p = 0.70)	0.06 (p = 0.68)	0.08 (p = 0.62)	0.04 (p = 0.82)	0.03 (p = 0.84)	0.08 (p = 0.63)	0.01 (p = 0.97)	0.31 (p = 0.16)
Pb	0.12 (p = 0.40)	−0.18 (p = 0.21)	**−0.33 (p = 0.03)**	−0.24 (p = 0.10)	**−0.33 (p = 0.03)**	−0.26 (p = 0.07)	−0.17 (p = 0.26)	−0.12 (p = 0.41)	−0.09 (p = 0.68)
Se	0.035 (p = 0.82)	0.05 (p = 0.72)	0.11 (p = 0.49)	0.12 (p = 0.44)	−0.01 (p = 0.97)	0.003 (p = 0.99)	−0.04 (p = 0.77)	−0.10 (p = 0.49)	0.34 (p = 0.13)
Sr	0.21 (p = 0.16)	−0.03 (p = 0.85)	−0.02 (p = 0.88)	−0.02 (p = 0.89)	0.05 (p = 0.73)	0.05 (p = 0.75)	0.05 (p = 0.72)	−0.01 (p = 0.96)	0.01 (p = 0.95)
Zn	0.02 (p = 0.91)	0.13 (p = 0.40)	0.29 (p = 0.06)	0.24 (p = 0.10)	0.27 (p = 0.08)	0.24 (p = 0.10)	0.19 (p = 0.22)	0.19 (p = 0.20)	0.16 (p = 0.49)

Rho values were obtained by Sperman's correlation test ($p < 0.05$).
BMD, bone mineral density.

present study showed significant associations between muscle fibers diameter and BMD of total femur and lumbar vertebrae L1-L4, as also reported in other studies (Patel et al., 2018).

Regarding metal exposure as risk factor for the onset and progression of bone-muscle diseases, a significant difference of Cd and Pb levels in both bone and muscle tissues among OP, Ope and CTR groups was observed. In bone, Cd levels in disease-affected groups (OP and Ope) resulted more than twice as high as in healthy subjects, while in muscle tissue they resulted even higher. In addition, both bone and muscle Cd level showed a negative association with BMD (total femur and femoral neck), *t*-score (total femur and femoral neck); and muscle Cd level showed a association with muscle fibers diameter. Several studies have reported a key role of Cd in the pathogenesis of osteoporosis, given its accumulation in patients' bone (Akesson et al., 2006; He et al., 2023; Scimeca et al., 2017). In fact, it is well known that Cd affects the proliferation and activity of osteoblasts, causing a decrease in alkaline phosphatase levels and Ca deposition (Al-Ghafari et al., 2019; Soudani et al., 2011). Ougier and colleagues estimated a higher incidence of osteoporosis related to Cd exposure in women, while a benchmark dose of 2.9 μg/g creatinine for urinary-Cd in osteoporotic women was assessed by Suwazono et al. (Ougier et al., 2021; Suwazono et al., 2010). In blood, Cd concentration above 1.0 μg/L in 243 participants and 0.5 μg/L in 121 women were considered risk factors for the occurrence of osteoporosis (Chung, 2022). Also, in a study on 3234 young adults aged 20–35 years, the negative association between blood-Cd and BMD was observed in women, but not in men (Lu et al., 2021). Huang et al. showed a association between blood-Cd and risk of osteoporosis in US population. Moreover, in this study, Cd accumulation was related to a decrease of muscle fibers diameter (Huang et al., 2023a). Accordingly, the association between urinary Cd level and the risk of sarcopenia was recently observed in 5790 participants (Liang et al., 2023). In Korean patients, high blood-Cd levels were associated with the prevalence of sarcopenia in elderly populations (Yoo et al., 2016). Also, previous studies demonstrated the dose-response relationship between blood or urine-Cd concentration and muscle weakness (García-Esquinas et al., 2020; Kim et al., 2016a). Accordingly, Cd exposure induced an intense inflammatory response in muscle and an up-regulating of pro-inflammatory cytokines, such as tumour necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) (He et al., 2023). The positive association between Cd level in muscle and age confirmed the accumulation in muscle tissues due to the long biological half-life of 10–30 years (Nordberg et al., 2015). Furthermore, Cd level was positively related to aging, leading to a deterioration in the quality of muscle tissue and consequently, to sarcopenia (Liang et al., 2023). Cadmium level was also negatively correlated with BMI, according to Padilla et al. and Huang et al. (Padilla et al., 2010; Huang et al., 2023b).

Lead concentration resulted higher in the OP group compared to both Ope and CTR group, in bone and muscle tissues, but controversial results on the ability of Pb to influence BMD and the risk of osteoporosis were reported (Dermience et al., 2015; Scimeca et al., 2017). In this study, a significant association between Pb bone level and BMD total femur and femoral neck was showed. Levels of Pb in bone measured by Chang et al. resulted comparable with those of our study and the lack of association between CTRs and Ope patients was also assessed (Chang et al., 2018). In other previous studies, Pb levels in plasma of OP subjects was observed 2-fold higher than in CTR subjects, and blood-Pb concentration was negatively associated with BMD (Campbell and Auinger, 2007; Chen et al., 2014; Visconti et al., 2023; Wei et al., 2021). Lim et al. showed that osteoporosis risk was positively correlated with Pb concentration (Lim et al., 2016). On the contrary, the association between Pb exposure and the increased risk of osteoporosis was found in men and not in women, as well as no significant association between blood-Pb and BMD was found (Alfvén et al., 2002; Huang et al., 2023a; Xu et al., 2023). In the present study, none association was found between level of Pb and muscle quality. Conversely, Pb exposure was linked to decreased muscular strength in young populations, as reported by Wu

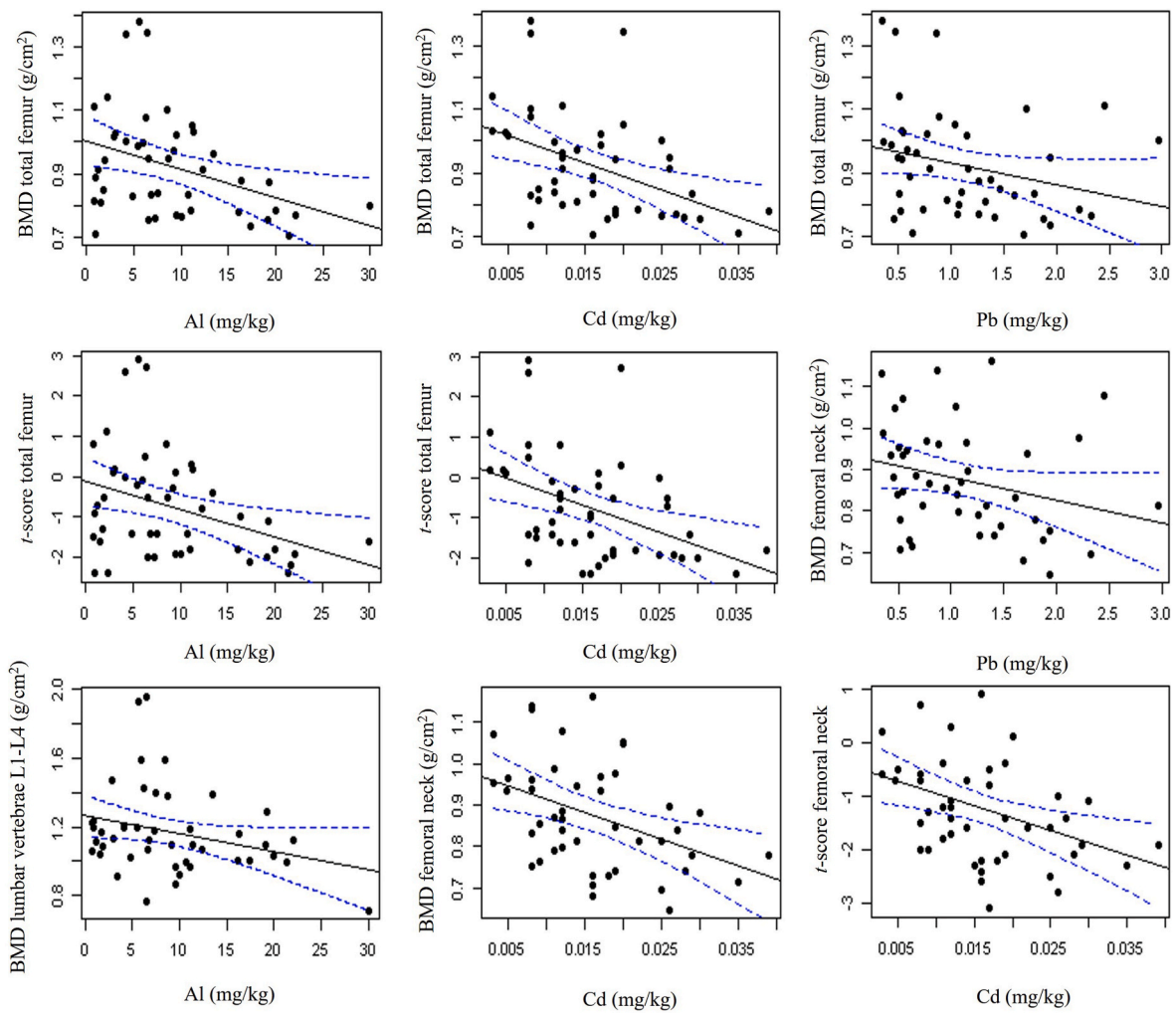

Fig. 4. Linear regression graphs of the significant negative association between Al, Cd, and Pb levels in bone, BMD and *t*-score. The dotted line represents 95% confidence intervals.

et al. and Nowak-Szczepanska et al., and to the increase risk of sarcopenia (Nowak-Szczepanska et al., 2022; Wu et al., 2022; Yoo et al., 2016).

Levels of Al in bone tissue of OP subjects was up to 2-fold higher than Ope and CTR groups. This result was confirmed by the negative association between Al and BMD of total femur and femoral neck, and between Al and BMD lumbar vertebrae. Aluminium levels found in bone of OP patients (10 mg/kg) correspond to the value for which pathological effects was ascertained by Klein and colleagues, and lower to those of 62 elderly patients (ranged 0.058–6.45 mg/kg) detected by Hellström et al. (Hellström et al., 2006; Klein, 2019). Although this study did not found association of Al with age, an exponential increase in Al content (in bone from 0.058 to 13.3 mg/kg) with increasing age from 16 to 98 years was observed by Hellström et al. (2005). It was proposed that Al osteotoxicity was due to the capability of transferrin receptor 1 (TfR1) on osteoblasts to bind this metal. Therefore, BMD levels close to osteoporosis threshold, could be related to the uptake of Al by osteoblasts (Cirovic and Cirovic, 2022). In accordance with these results, in plasma of 4924 participants from Dongfeng-Tongji cohort, Al resulted significantly associated with the risk of osteoporosis both in males and females (Xu et al., 2023). On the contrary, Di et al. and Visconti et al. showed no significant difference in Al plasma level between OP and CTR groups (Di et al., 2023; Visconti et al., 2023).

Regarding Co, in muscle tissue it was found negatively associated with BMD of total femur, *t*-score of total femur and femoral neck, and muscle fibers diameter, and also positively with age. Cd and Co exposure resulted in an acceleration of cellular senescence and in aging of organism, aggravating muscle qualitative state, and ultimately leading to sarcopenia (Liang et al., 2023). As reported by Laumonier et al. the exposure of human myoblasts to Co^{2+} ions, appeared to influence cell viability and myogenic differentiation (Laumonier et al., 2020). In addition, results on 1615 subjects showed that Co was associated with a significant increase in mobility impairment and sarcopenia risk (Huang et al., 2023a; Lang et al., 2009).

In our study, we found no statistical differences among the three groups of patients in terms of Cr levels in either bone or muscle, although its concentration is 3 times higher in the OP group than in Ope and CTR subjects. Noteworthy, we observed that Cr accumulation in muscle tissue was negatively associated with muscle fibers diameter, which was reduced in OP patients. Accordingly, Scimeca et al. assessed bone Cr accumulation in OP than CTR subjects, while Di and colleagues did not found association between Cr and osteoporosis (Di et al., 2023; Scimeca et al., 2017).

It is known that also other metals may play a role in the pathogenesis of osteoporosis and osteopenia. We found that Hg accumulation was negatively associated with muscle fibers diameters, although no statistically significant difference in Hg levels in muscle and bone tissues among groups was observed. Similarly, also Huang et al. did not observe association with osteoporosis and blood-Hg levels (Huang et al., 2023b). Conversely, lower blood-Hg level was reported as a risk factor of

Table 5
Association between level of metals in muscle tissue samples and subject characteristics.

Metal	Age	BMI	BMD total femur	*t*-score total femur	BMD femoral neck	*t*-score femoral neck	BMD lumbar vertebrae L1-L4	*t*-score lumbar vertebrae L1-L4	Muscle fibers diameter
Al	0.10 (p = 0.54)	−0.24 (p = 0.17)	−0.15 (p = 0.40)	−0.07 (p = 0.69)	0.17 (p = 0.93)	0.05 (p = 0.77)	−0.08 (p = 0.65)	0.01 (p = 0.97)	−0.08 (p = 0.70)
As	0.29 (p = 0.07)	−0.05 (p = 0.76)	−0.01 (p = 0.94)	−0.05 (p = 0.76)	−0.003 (p = 0.99)	−0.06 (p = 0.75	**0.39 (p = 0.03)**	0.32 (p = 0.06)	−0.10 (p = 0.62)
Cd	**0.47 (p = 0.002)**	**−0.36 (p = 0.03)**	**−0.47 (p = 0.01)**	**−0.50 (p = 0.002)**	**−0.42 (p = 0.02)**	**−0.42 (p = 0.01)**	0.09 (p = 0.63)	0.15 (p = 0.41)	**−0.44 (p = 0.02)**
Co	**0.48 (p = 0.002)**	−0.03 (p = 0.87)	**−0.34 (p = 0.05)**	**−0.43 (p = 0.01)**	−0.25 (p = 0.16)	**−0.39 (p = 0.02)**	0.20 (p = 0.26)	0.13 (p = 0.47)	**−0.46 (p = 0.01)**
Cr	0.22 (p = 0.17)	−0.13 (p = 0.47)	−0.05 (p = 0.78)	−0.16 (p = 0.36)	−0.18 (p = 0.32)	−0.24 (p = 0.17)	0.16 (p = 0.38)	0.08 (p = 0.67)	**−0.53 (p = 0.004)**
Cu	0.01 (p = 0.96)	0.01 (p = 0.97)	−0.02 (p = 0.92)	−0.05 (p = 0.79)	−0.25 (p = 0.16)	−0.24 (p = 0.16)	0.18 (p = 0.33)	0.23 (p = 0.18)	−0.20 (p = 0.30)
Hg	0.25 (p = 0.11)	−0.03 (p = 0.85)	−0.15 (p = 0.42)	−0.16 (p = 0.36)	0.06 (p = 0.76)	0.06 (p = 0.74)	0.28 (p = 0.63)	0.24 (p = 0.17)	**−0.48 (p = 0.01)**
Mn	**0.32 (p = 0.05)**	−0.14 (p = 0.42)	−0.10 (p = 0.59)	−0.10 (p = 0.59)	−0.11 (p = 0.56)	−0.12 (p = 0.49)	0.20 (p = 0.26)	0.16 (p = 0.36)	−0.15 (p = 0.40)
Ni	0.26 (p = 0.10)	−0.14 (p = 0.41)	−0.11 (p = 0.56)	−0.22 (p = 0.20)	0.03 (p = 0.87)	−0.10 (p = 0.56)	0.11 (p = 0.54)	0.03 (p = 0.85)	−0.29 (p = 0.13)
Pb	0.27 (p = 0.10)	−0.04 (p = 0.80)	−0.10 (p = 0.58)	−0.10 (p = 0.58)	−0.28 (p = 0.12)	−0.29 (p = 0.10)	0.07 (p = 0.69)	0.16 (p = 0.37)	−0.26 (p = 0.17)
Se	0.15 (p = 0.34)	−0.20 (p = 0.24)	−0.08 (p = 0.67)	−0.17 (p = 0.34)	−0.25 (p = 0.17)	−0.33 (p = 0.06)	0.12 (p = 0.50)	0.06 (p = 0.73)	−0.28 (p = 0.15)
Sr	0.18 (p = 0.26)	0.14 (p = 0.44)	0.16 (p = 0.37)	0.12 (p = 0.55)	0.26 (p = 0.14)	0.20 (p = 0.25)	0.01 (p = 0.95)	−0.09 (p = 0.61)	−0.04 (p = 0.84)
Zn	0.04 (p = 0.79)	−0.13 (p = 0.46)	−0.06 (p = 0.72)	−0.13 (p = 0.47)	−0.05 (p = 0.80)	−0.06 (p = 0.74)	0.08 (p = 0.66)	0.10 (p = 0.59)	−0.28 (p = 0.16)

Rho values were obtained by Sperman's correlation test ($p < 0.05$).
BMD, bone mineral density.

osteopenia or osteoporosis, and higher blood-Hg was reported as a risk factor for sarcopenia by Yoo et al. (Cho et al., 2012; Yoo et al., 2016). Moreover, Kim et al. showed negatively association between blood-Hg and BMD of femoral neck in a Korean population (Kim et al., 2016b).

No association was observed between Mn and bone or muscle quality, but a positive association between age and Mn level in muscle was detected. Similar outcome was found for blood-Mn level and osteoporosis (Huang et al., 2023b; Odabasi et al., 2008). On the contrary, a negative association between blood-Mn and BMD, especially of femoral neck, was observed in 9732 adult subjects (Wang et al., 2022). The study by Li and colleagues showed that population of retired female workers was characterized by an increased risk of osteoporosis occurrence, due to long-term occupational exposure to Mn (Li et al., 2020). Moreover, in a recent study on 4957 individuals, the chronic inflammation markers as alkaline phosphatase seemed to mediate the relationship between Mn exposure and the prevalence of sarcopenia (Huang et al., 2024).

The first limitation of the study is represented by the small number of subjects sampled. Above all, the small size of the OP patients group is due to the difficulty of recruiting patients with osteoporosis who were not concomitantly suffering from comorbidities, or who were undergoing drug therapy (exclusion criteria). In addition, the selection of CTR subjects with comparable age to OP and Ope patients is inherently difficult, considering the invasive surgery required to collect the bone and muscle samples analyzed in this study. Noteworthy, other confounding variables that could affects the metals content in bone and muscle tissues, such as diet and life-style, were not included. Further larger samples are needed to perform confounding factors analysis and confirm the results obtained from this pilot study. Finally, the standard diagnostic procedure for sarcopenia, usually measured with physical performance tests such as the Short Physical Performance Battery (SPPB), could not be performed on the subjects because functional limitations after surgery. Therefore, only histomorphometric analysis of muscle fibers diameter was performed to assess muscle quality.

5. Conclusions

This study evaluated the potential association between metal levels in bone and muscle samples and the degeneration of both tissues in osteoporotic diseases. Bone tissue degeneration was evaluated in terms of decreased BMD, while muscle tissue degeneration was assessed by measuring muscle fibers diameter. Although it is well known that exposure to metals is an important risk factor for osteoporosis, the quantification of metal level in human bone and muscle samples is still poorly investigated. Therefore, this study highlights how some metals may exert a deleterious effect on the quality of bone and muscle tissues, potentially contributing to the progression of osteoporotic disease. Given the importance of metal accumulation in the pathophysiology of osteoporosis, further studies on metal exposure effects should be advisable, so that early intervention can be taken before bioaccumulation of metals can further impair bone and muscle quality and worsen musculoskeletal health status, preventing the disease progression.

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CRediT authorship contribution statement

Beatrice Battistini: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Chiara Greggi:** Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **Virginia Veronica Visconti:** Writing – original draft. **Marco Albanese:** Formal analysis, Data curation. **Alessandra Messina:** Methodology. **Patrizia De Filippis:**

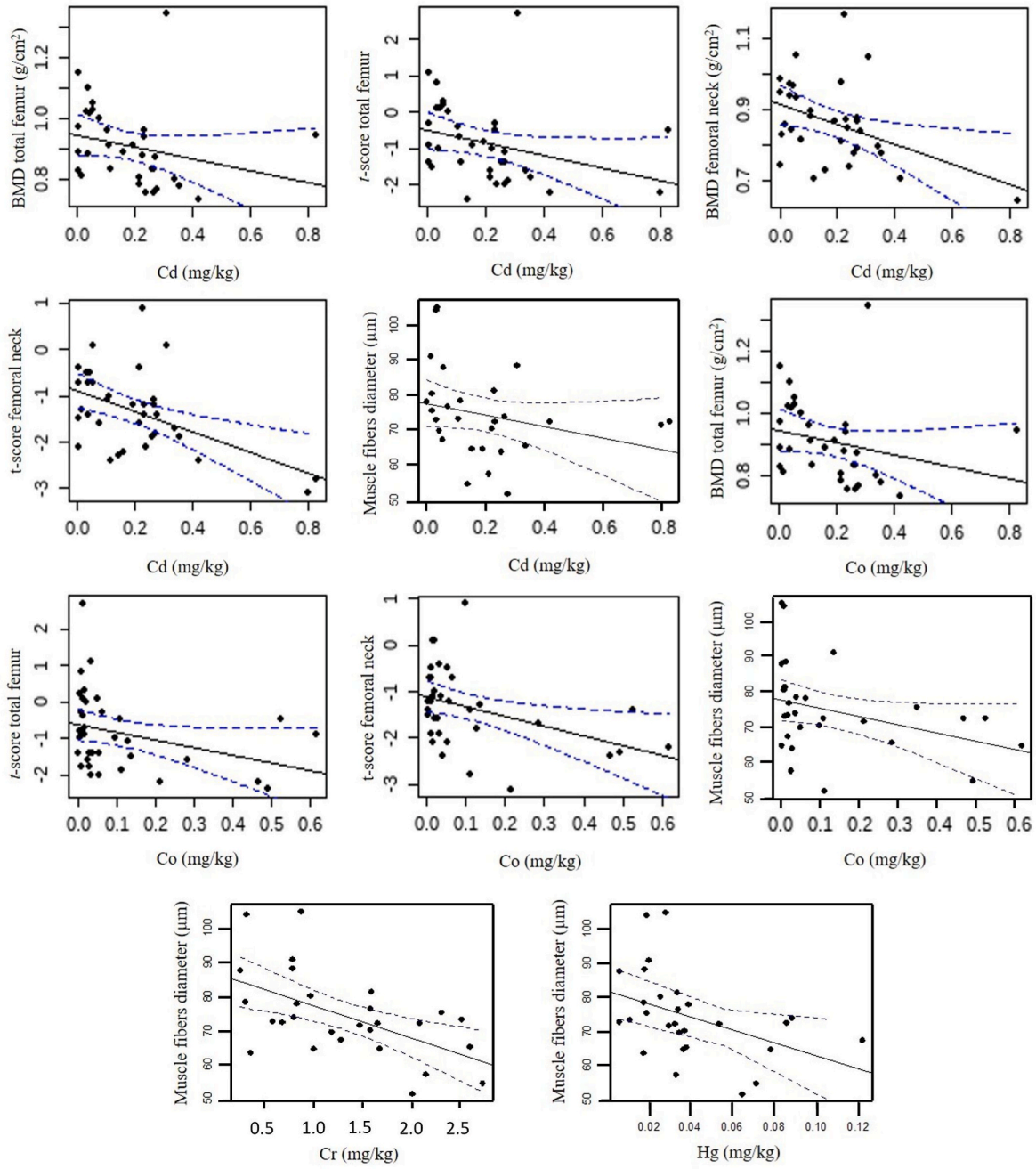

Fig. 5. Linear regression graphs of the significant negative association between Cd, Co, Cr, Hg levels in muscle, BMD, *t*-score and muscle fibers diameter. The dotted line represents 95% confidence intervals.

Methodology. **Beatrice Gasperini:** Investigation. **Angela Falvino:** Methodology, Investigation. **Prisco Piscitelli:** Investigation, Conceptualization. **Leonardo Palombi:** Investigation, Conceptualization. **Umberto Tarantino:** Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.118514.

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