Impaired skeletal health and neuromuscular function among amphetamine users in clinical treatment

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MINI ABSTRACT

This study examined musculoskeletal health in amphetamine users, compared with healthy age-matched controls. We show that amphetamine users have reduced bone mass at several skeletal sites, and attenuated maximal muscle strength and force development capacity in the lower extremities.

ABSTRACT

Amphetamine use may cause poor bone quality and elevated risk of osteoporosis. **Purpose:** To investigate whether amphetamine users exhibit reduced regional and whole body bone mineral density (BMD), altered bone metabolism, and how muscle function may relate to the patient groups' skeletal health. **Methods:** We assessed hip, lumbar spine and whole body BMD, and trabecular bone score (TBS) by dual X-ray absorptiometry (DXA); bone metabolism markers in serum, and maximal strength and force development capacity in 36 amphetamine users, (25men, 30±7yr; 11women 35±10yr) and in 37 healthy controls (23 men, 31±9yr; 14 women, 35±7yr). **Results:** Whole body BMD was lower in amphetamine users (8% in males and 7% females, $p<0.01$), as were BMD at the total hip and sub-regions of the hip (9-11% in men; 10-11% in women, $p<0.05$). Male users had 4% lower TBS ($p<0.05$) and higher serum level of type 1 collagen amino-terminal pro-peptide ($p<0.01$). This coincided with reduced lower extremity maximal strength of 30% (males, $p<0.001$) and 25% (females, $p<0.05$), and 27% slower muscular force development in males compared to controls ($p<0.01$). **Conclusions:** These findings demonstrate that amphetamine users suffer from a generalized reduction in bone mass, which was associated with attenuated maximal muscle strength and force development capacity in the lower extremities.

Key words: Addiction, Rehabilitation, Physical capacity, Body composition, One repetition maximum, Rate of force development

INTRODUCTION

It is well documented that patients suffering from amphetamine abuse have an impaired physical and mental health [1-4]. The etiology of the health problems is certainly multifactorial, but there are indications that impairment of skeletal muscle and bone may be important contributors. Previously, we and others have shown that amphetamine users have impaired physical function and muscular strength [5, 6], likely due to a lamentable lifestyle with little physical activity. Reduced muscle function is typically accompanied by attenuated bone health [7, 8]. Only a few studies have reported evidence of an association between long-term amphetamine use and impaired bone health [9-11]. It also remains unclear how skeletal health relate to muscular properties in amphetamine abusers.

One study reported that bone quality, measured by Achilles ultrasound bone densitometer, was reduced among male methamphetamine users [9]. It has also been reported that methamphetamine users displayed lower bone mineral density (BMD) at the lumbar spine, measured by dual X-ray absorptiometry (DXA), and higher prevalence of osteoporosis compared with age-matched controls [10]. Although these are indications of a poor skeletal health, it is not clear whether amphetamine abusers exhibit a systemic bone loss. Additionally, none of these studies included analyses of bone metabolism markers in blood, and muscle strength and function were not addressed [9, 10]. The mechanisms by which amphetamines may affect bone health are generally unclear. One study in mice showed that methamphetamine had dose-dependent effects on bone turnover, exhibiting suppressed bone turnover when given at a high dosage, while increasing osteoblast number at a lower dose [11]. The latter study suggests that amphetamines may directly affect skeletal properties independent of muscle alterations.

Skeletal health is also strongly related to lifestyle and physical capacity [7, 8]. In line with this, a close relationship between skeletal health and neuromuscular function has been demonstrated by several studies [12-14]. Neuromuscular performance has been reported to strongly predict bone quality in women [12]. Additionally, we and others have demonstrated that improvements in neuromuscular function by exercise, is typically accompanied by increased bone mass in adults [13-15]. There is generally a lack of knowledge regarding neuromuscular function among amphetamine users. Nevertheless, as a strong stimulant of neurotransmitters, amphetamines have been shown to induce a diverse range of neurotoxic side effects such as oxidative stress [16], mitochondrial damage [17] and inflammation in the central nervous system (CNS) [18]. Amphetamines have also been reported to cause nerve cell apoptosis [19, 20], indicating a direct involvement in neuronal damage. These findings suggest that amphetamine abuse may cause deteriorated neuromuscular function in humans.

Skeletal health, and muscle strength and function are important determinants of physical function [21] and even all-cause mortality [22], thus potentially affecting both quality of life and overall life expectancy in individuals with severe amphetamine dependency. Identifying new potential risk factors related to physical health in amphetamine users would be advantageous for offering a more profound rehabilitation regime. Thus, the aim of the current study was to assess skeletal properties, along with muscle strength and neuromuscular force capacity in the lower extremities of amphetamine users in clinical treatment, compared with age-matched controls.

METHODS

We recruited amphetamine users from the University hospital drug clinic, St. Olav's hospital, Trondheim, Norway. Bone mineral content (BMC), BMD, trabecular bone score (TBS) and body composition were assessed by DXA analyses. Serum levels of bone metabolism markers and 25-hydroxy vitamin D_3 (25OH D) were analyzed by immunoassays, while maximal muscle strength and rate of force development (RFD) in the lower extremities were determined as measures of muscle function.

Subjects

After signing written informed consent, 36 inpatients, 25 males (age 30±7yr; weight 79.4±12.7kg; height 180±7cm) and 11 females (age 35±10yr; weight 71.2±19.1kg; height 164±7cm) volunteered to participate in the study. All subjects had a diagnosis of substance use disorder, according to WHO diagnostic system (ICD-10: F10-19), and had amphetamine as the predominant drug. Seventeen of the males and eight of the females were current cigarette smokers. The patients' characteristics, substance use and medications are given in table 1. An age- and sex-matched control group consisting of 23 males (age 31±9yr; weight 82.4±12.3kg; height 183±7cm) and 14 females (age 35±7yr; weight 68.9±7.7kg; height 171±5cm), were recruited among students and employees at the hospital. In the control group, one male and four females were current cigarette smokers. Exclusion criteria for both groups were injuries or other medical conditions that prevented them from carrying out the physical tests. The regional medical ethics committee (REK-nord) approved the study, and it was carried out in accordance with the Declaration of Helsinki.

Substance use measurements

EuropASI (Addiction Severity Index, European adaption of The American 5th edition [23]) was applied to quantify the extent and history of substance use and age at debut.

Dual X-ray absorptiometry (DXA) assessments

Using established procedures [14], bone mineral content (BMC) and areal BMD at the lumbar spine (L1–L4), hip region and whole body were measured by DXA applying Hologic (Discovery, S/N 83817). The coefficients of variations for BMD were 1.1% at the lumbar spine, 1.3% at the total hip, and 1.5% at the femoral neck. Osteoporosis was defined according to WHO as a T-score $\leq$ -2.5 SD below the average BMD of young adults and osteopenia as a T-score between -1 and -2.4 SD. Fat mass, lean mass and total body mass were also assessed by whole body DXA. Trabecular bone score (TBS) which is an estimate of bone quality was measured at the lumbar spine using TBS iNsight® Software version 1.8 (Med-Imaps, Pessac, France). Coefficient of variation for the TBS analyses has been reported to be less than 2% [24]. All DXA measurements were carried out by a certified technician at the Department of Endocrinology at St. Olav's Hospital, Trondheim, Norway.

Immunoassays

Serum levels of 25-hydroxy vitamin D3 were determined by radioimmunoassay (RIA) (DIAsource, Louvain-la-Neuve, Belgium), using the manufacturer's procedure and controls. Serum level of the bone formation marker type 1 collagen amino-terminal propeptide (P1NP) was determined by radioimmunoassay (Orion Diagnostica, Espoo, Finland). The detection limit was 2mg $\cdot$ L^{-1}, and inter- and intra-assay variations were 6.5 and 7.0%, respectively.

Concentration of the bone resorption marker type 1 collagen C breakdown products (CTX) was determined by a Serum CrossLaps enzyme-linked immunosorbent assay (ELISA) (Nordic Bioscience Diagnostics A/S, Herlev, Denmark). The detection limit was 0.020ng · mL^{-1}, and inter- and intra-assay variations were 6.5 and 5.1%, respectively. Detection limits and variances were determined by the manufacturers.

Maximal muscle strength and rate of force development

Maximal strength was measured as one repetition maximum (1RM) in a hack squat machine (impulse Fitness IT7006, Shandong, China). 1RM was obtained by repeating the lift, down to a 90° knee joint angle, with increasingly loads of 5-10 kg until the subjects were not able to complete the lift. The highest load was obtained in 6-8 lifts and set as 1RM.

Rate of force development (RFD) was measured using a force plate (9286AA, Kistler, Switzerland), attached to the hack squat machine. The subjects performed three attempts with a load corresponding to 80% of 1RM, and the best attempt was recorded as their RFD. Between each trial there was a 2-3 minutes rest. Importantly, to ensure maximal mobilization of the nervous system, the subjects were instructed to aim for maximal intended velocity in the concentric phase during the squat movement. Data were registered at 2000Hz (Bioware v3.06b, Kistler, Switzerland). RFD was measured between 10% and 90% of peak force in the concentric phase of the lift. All strength measurements were performed in accordance with previously established procedures [14].

Statistics

The statistical analyses were carried out using the SPSS, version 20, software program (Chicago, USA), and figures were made using the software GraphPad Prism 5 (San Diego, USA). The data were tested for normal distribution using Levene's test of homogeneity of variances. Differences between groups were analyzed by one-way analysis of variance (ANOVA), using Tukey post hoc test to correct for multiple testing, or by Mann-Whitney U test when normal distribution could not be assumed. Correlations between muscle function and BMD were established using linear Pearson correlation regression analysis. Statistical significance was accepted at $P < 0.05$. Data are presented as mean ± SD unless otherwise stated.

RESULTS

Background data

Twenty-five male and 11 female amphetamine-using patients, a gender distribution similar to what is typically seen in the treatment clinic, were included in the study. The drug debut age, secondary drug and years of amphetamine use are given in Table 1. Amphetamine users and controls were age-matched to avoid age related differences in musculoskeletal properties. Whereas no difference in body weight between the groups was observed, body length was significantly lower in the female amphetamine users than the controls (Table 2). No differences in baseline characteristics that could interfere with musculoskeletal properties were observed between the groups. There were twenty-five current smokers in the amphetamine group (seventeen males and eight females), and five in the control group (one male and four females). Nine of the participants (eight in the amphetamine group and one in the control group) were using prescribed medicines that are known to affect bone (i.e. proton pump inhibitors, anti-epileptic and selective serotonin re-uptake inhibitors (SSRI), and glucocorticoids)[25]. Whilst all thirty-six patients completed the DXA scans, one male and two females withdrew from the study, without giving specific reasons, before carrying out the strength measurements.

Anthropometric parameters

There were no significant differences in total body mass (kg) or fat mass (kg) between the amphetamine group and control group. However, amphetamine-using males had a significantly lower lean muscle mass (10%) and lower extremity lean mass (13%) compared to controls. Also female patients expressed a significantly reduced lower extremity lean mass (14%) compared to their healthy counterparts (Table 2). As a consequence, both male and

female patients had a significantly higher fat mass percentage (19%, males, 18%, females) (Table 2).

Bone mineral content and bone mineral density

Male patients exhibited significant reductions in BMC at the femoral neck (10%), trochanter (14%), intertrochanteric hip (20%), total hip (18%) and total body (13%) compared to the control group (Table 3). Similar observations were made in the female patients with significantly lower BMC measured at the total hip (21%) intertrochanteric hip (26%) and total body (13%) (Table 3).

Amphetamine-dependent males displayed significant reductions in whole body, total hip and femoral neck BMD by 8%, 10% and 9%, respectively. A significantly lower BMD was also apparent for trochanter (9%), intertrochanteric hip (10%) and Ward's triangle (11%), whilst no significant differences between the groups were observed for lumbar spine BMD (Table 3). Female patients exhibited similar impairments as the male patients. Total hip and whole body BMD were significantly reduced by 11% and 7%, respectively, whilst femoral neck BMD tended ($p=0.066$) to be reduced. Additionally, intertrochanteric hip BMD was also 10% lower, and significantly different from the control group (Table 3).

Z-scores were significantly lower in amphetamine users than in controls at the whole body (-0.79±0.94 vs. 0.28±0.83 in men and -0.78±0.65 vs. 0.32±0.91 in women), total hip (-0.26±0.76 vs. 0.49±0.76 in men and -0.21±0.78 vs. 0.66±0.76 in women), and femoral neck (-0.36±0.79 vs. 0.30±0.86 in men and -0.47±0.73 vs. 0.28±0.99 in women). Mean T-scores

were all below zero for the amphetamine users. Whole body T-scores were -0.79±0.96 (men) and -0.77±0.68 (women). Specifically, 15 out of 25 males, and 4 out of 11 females, had osteopenia with a whole body T-score between -1.0 and -2.5.

Trabecular bone score

Male amphetamine users had significantly lower TBS (4%) at the lumbar spine compared to controls, while female users tended (p=0.074) to have lower TBS (5%) (Table 3).

Serum markers of bone metabolism

The males in the amphetamine group had significantly higher serum levels of P1NP (48%) than controls. P1NP levels were 23% higher also in females, although not significantly (p=0.104). No significant differences were observed in serum CTX, or 25OH D level (Table 3).

Maximal muscle strength and rate of force development

Male amphetamine users differed significantly in all strength measurements compared with the controls, exhibiting a 30% lower maximal muscular strength (183±37kg in the control group vs. 128±38kg in the amphetamine group), a 27% lower rate of force development (1839±462N·s^{-1} in the control group vs. 1339±576N·s^{-1} in the amphetamine group) and a 19% lower peak force (2525±358N in the control group vs. 2040±371N in the amphetamine group) (figure 1). As for the males, female patients displayed a 25% lower maximal muscular strength (105±30kg in the control group vs. 79±29kg in the amphetamine group) (figure 1).

However, rate of force development and peak force were not significantly different from the control group.

Correlation between BMD and muscle function

1RM was significantly associated with BMD at the total hip ($r=0.504$, $p<0.001$) and femoral neck ($r=0.467$, $p<0.001$). Also RFD correlated significantly with total hip and femoral neck BMD, $r=0.470$ and $r=0.426$, respectively ($p<0.001$).

DISCUSSION

Since it is unclear if amphetamine dependence impairs bone health, this study sought to investigate bone mass characteristics and markers of bone metabolism in amphetamine- using patients. We also wanted to examine if a possibly attenuated bone health was associated with reductions in muscle strength and muscle force development characteristics. The main findings were that amphetamine users displayed reduced bone mass at the hip and whole body, along with impaired bone quality at the lumbar spine, and that this coincided with reduced maximal muscle strength and neuromuscular force capacity.

Both female and male amphetamine users exhibited reduced BMC and BMD. BMC was significantly lower than in the control group at most of the skeletal regions, as was BMD with reductions of 8-12%. Furthermore, both male and female amphetamine users had low Z-scores compared to controls, being yet another manifestation of reduced bone mass. Our results expand the observations from a previous study showing male methamphetamine users to have a lower BMD at the lumbar spine [10]. Although lumbar spine BMD was not significantly lower in our study, TBS was significantly reduced in both male and female amphetamine users, indicating impaired bone quality. TBS is recognized as a predictor of vertebral fractures as well of osteoporotic fractures in general [26]. Approximately half of the patients in our study had osteopenia, whilst many of the amphetamine users in the study by Kim et al. had developed osteoporosis [10]. Taken together, our and previous data [9, 10] suggest that amphetamine users have compromised skeletal health.

Interestingly, amphetamine users had elevated serum levels of the bone formation marker P1NP compared to the control subjects, and also higher than typically observed in men and

women at a similar age [27]. CTX in serum did not differ between the groups, indicating that bone resorption was unaffected. As this finding coincided with low bone mass, it may reflect a compensatory effect. Our findings are in line with one study showing that serum levels of the bone formation markers alkaline phosphatase and osteocalcin increased after eight weeks of methamphetamine administration in mice [11]. This study also revealed increased number of osteoblasts at the distal femur of mice given methamphetamine, but without any effect on bone mass or structure [11]. In the same study, however, mice given a higher dosage of methamphetamine displayed a reduced bone turnover indicating differential effects on bone metabolism dependent on dose. Increased serum level of P1NP has also been associated with vitamin D deficiency in patients with osteomalacia [28, 29]. The participants in our study displayed relatively low levels of circulating vitamin D [30, 31]. However, this cannot alone explain the increased P1NP level in the amphetamine group as no difference in vitamin D status between the groups was observed.

Amphetamines may affect bone metabolism through their actions on neurotransmitters and the CNS. Amphetamine has been shown to interact with the neuroendocrine system, including the ß-adrenergic receptors (ßAR) [32]. Activation of the ß-2 adrenergic receptor (ß2AR) is known to increase osteoclastogenesis and to promote bone loss mediated through altered gene expression of signaling proteins like receptor activator of nuclear factor kappa-B ligand and interleukin-6 [33-35]. It has also been reported that ßAR activation acutely inhibits the proliferation of osteoblasts, leading to reduced bone formation [36]. Therefore, a possible mechanism by which amphetamines may contribute to reduce bone mass is through altering the gene expression of cytokines involved in osteoclast and osteoclast activity. Whether these mechanisms contribute to the bone loss observed in long-term amphetamine abusers remain unclear.

Reduced muscle function may be one of the mediators of the impaired bone health in our study. 1RM was 30% and 25% lower in male and female amphetamine users, respectively, whereas neuromuscular force capacity, expressed as RFD, was reduced by 27% in the male amphetamine group. Maximal muscle strength and force development capacity also correlated well with BMD at the hip. These findings are in accordance with several other studies [12-14], demonstrating skeletal properties to mirror neuromuscular activity and function. Accordingly, RFD has been shown to strongly predict bone quality in both pre- and postmenopausal women [12]. RFD is also recognized as an important factor accounting for walking ability, and the capacity for conducting everyday tasks [37], thus potentially having a severe impact on quality of life in general. The muscle strength reductions among the amphetamine users correspond to what is typically observed at 30-40 years of age [38], and are closely linked to fall and fracture risk [39], every day physical function [39, 40], and even all-cause mortality [22]. The observed reductions in muscular strength and bone mass may be influenced not only by the amphetamine use, but could also be a result of inactivity.

Muscle strength is dependent not only on neural factors, but also relies on muscle mass. In this study amphetamine users had an unfavorable body composition, with less lean mass and more fat mass. The 13% lower lean mass in the lower extremities of the amphetamine users is in accordance with the reduced muscular strength that was observed. Loss of lean mass is usually caused by low levels of muscle activity, which has been advocated as an essential cause of age-related bone loss [41, 42]. Muscle activity is mainly known to affect bone metabolism by causing mechanical strain [43]. Recent studies have also shown that skeletal muscle releases endocrine factors that can directly affect bone metabolism at the molecular

level [44]. Therefore, reduced muscle mass could be another factor contributing to the bone loss observed in the amphetamine users.

Life style factors also clearly contribute to inferior musculoskeletal health in amphetamine users. The impaired muscle function is likely a result of a sedentary life style overall. Furthermore, smoking was more frequent in the amphetamine group, and a high proportion had additional substance use. The prevalence of prescribed medicines was also high in this group, and some of them used medications that are known to affect bone metabolism [25]. The impaired skeletal health and neuromuscular function observed in amphetamine users may result in sarcopenia and osteoporotic fractures, and subsequently reduce everyday function and quality of life.

In conclusion, this is the first report showing that amphetamine users suffer a systemic reduction of bone mass. These reductions were accompanied by a higher serum level of bone formation marker, suggesting a compensatory response. Finally, the impaired bone health was associated with reduced muscle strength and force development characteristics, which may play a mediating role. In combination, these findings imply that rehabilitation of amphetamine abusers should include strength training that aims to counteract the negative musculoskeletal effects.

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FIGURE LEGENDS

Figure 1

Differences between the groups in maximal muscle strength (A) and force capacity (B) in the lower extremities. Data are presented as mean and SD. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.

Table 1: Patients' history of amphetamine use, secondary drug dependence and medical use.

	Males (n = 25)	Females (n = 11)
Amphetamine use (yr)	12 ± 7	12 ± 6
Drug use debut (age)	14 ± 2	15 ± 2
Secondary drug:		
Alcohol	4	2
Heroin	2	1
Cocaine	2	-
Benzodiazepines	3	1
Cannabis	13	7
Hallucinogen	1	-
Prescribed medicines for symptoms:		
ADHD	-	1
Allergies	3	3
Anxiety	10	6
Arthritis	3	-
Asthma/COPD	3	1
Depression	4	2
Epilepsy	3	1
Migraine	1	4
Hypertension	4	1
Infections	1	2
Schizophrenia/Bipolar	6	4
Substitutional treatment	3	2
Other	3	1

Data are presented as mean ± SD. Prescribed medicines in substitutional treatment: Methadone and Suboxone. Other (prescribed medicines): Skin disorder; pain; inflammation**.**

Table 2. Participant characteristics and body composition

	Male (n=48)		Female (n=25)	
	Control (n=23)	**Amphetamine (n=25)**	**Control (n=14)**	**Amphetamine (n=11)**
Age (y)	30.5 ± 9.4	30.0 ± 6.8	34.9 ± 7.3	34.9 ± 9.7
Height (cm)	183.3 ± 6.7	180.0 ± 6.4	171.8 ± 4.8	163.9 ± 6.8**
Body mass (kg)	82.4 ± 12.3	79.4 ± 12.4	69.0 ± 7.7	71.2 ± 19.1
BMI (kg·m-2)	24.5 ± 3.0	24.5 ± 3.3	23.4 ± 3.2	26.6 ± 7.1
Fat mass (kg)	12.9 ± 4.9	15.0 ± 5.7	18.6 ± 4.9	22.1 ± 8.0
Fat mass (%)	15.2 ± 4.0	18.1 ± 4.2*	26.1 ± 4.5	30.7 ± 3.9*
Lean mass (kg)				
Whole Body	67.8 ± 8.4	61.0 ± 13.7*	49.3 ± 4.0	46.6 ± 9.8
Lower extremity	23.2 ± 2.6	20.3 ± 3.0**	16.5 ± 1.6	14.2 ± 3.0*

Data are presented as mean ± SD. * = significant difference between groups, $p < 0.05$. ** $p < 0.01$.

Table 3. BMD, BMC, TBS and serum markers

	Male (n=48)		Female (n=25)	
	Control (n=23)	**Amphetamine (n=25)**	**Control (n=14)**	**Amphetamine (n=11)**
BMC (g)				
Lumbar spine (L1-L4)	74.26 ± 12.44	69.08 ± 10.32	66.79 ± 13.95	61.30 ± 8.58
Femoral neck	5.49 ± 0.73	4.95 ± 0.72*	4.32 ± 0.76	3.90 ± 0.55
Trochanter	11.04 ± 2.01	9.44 ± 1.83**	7.80 ± 1.64	6.85 ± 1.43
Intertrochanteric hip	38.93 ± 6.77	30.99 ± 4.54***	26.93 ± 3.20	20.05 ± 3.27***
Total hip	55.54 ± 8.63	45.38 ± 6.24***	39.06 ± 4.76	30.80 ± 4.92**
Ward's triangle	0.895 ± 0.177	0.844 ± 0.164	0.901 ± 0.214	0.793 ± 0.173
Whole body	2396.53 ± 318.07	2085.94 ± 288.00***	1810.84 ± 260.74	1574.92 ± 185.6**
BMD ($g \cdot cm^{-2}$)				
Lumbar spine (L1-L4)	1.046 ± 0.100	1.009 ± 0.089	1.065 ± 0.144	1.002 ± 0.087
Femoral neck	0.941 ± 0.121	0.858 ± 0.108*	0.855 ± 0.115	0.775 ± 0.085
Trochanter	0.774 ± 0.091	0.703 ± 0.083**	0.719 ± 0.105	0.673 ± 0.068
Intertrochanteric hip	1.271 ± 0.126	1.146 ± 0.142**	1.178 ± 0.112	1.058 ± 0.121*
Total hip	1.093 ± 0.112	0.981 ± 0.115**	1.007 ± 0.097	0.901 ± 0.095*
Ward's triangle	0.796 ± 0.145	0.707 ± 0.129*	0.746 ± 0.142	0.665 ± 0.115
Whole body	1.094 ± 0.088	1.006 ± 0.080***	0.971 ± 0.074	0.903 ± 0.045**
TBS				
Score	1.462 ± 0.074	1.401 ± 0.090*	1.484 ± 0.102	1.410 ± 0.084
Serum markers				
25OH D ($nmol \cdot L^{-1}$)	64.64 ± 18.57	67.61 ± 24.03	75.22 ± 23.07	63.29 ± 17.10
P1NP ($\mu g \cdot L^{-1}$)	50.14 ± 11.22	96.08 ± 36.38**	54.03 ± 15.33	69.60 ± 27.50
CTX ($ng \cdot mL^{-1}$)	0.49 ± 0.25	0.47 ± 0.26	0.41 ± 0.27	0.37 ±0.20

Data are presented as mean ± SD. SUD = substance use disorder group, REF = reference group. BMD = bone mineral density, BMC = bone mineral content, TBS = trabecular score, 25OH D = 25-hydroxy vitamin D_3, P1NP = type 1 collagen amino propeptide, CTX = type 1 collagen C, * = significant difference between groups, $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure

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