Neuropsychiatric Disease and Treatment

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ORIGINAL RESEARCH

The Impact of Depression on Detrimental Changes in Bone Microstructure in Female Mice

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Background: Several clinical studies have examined the connection between depression and bone loss, but the cause-and-effect relationship between the two conditions, especially in animal models, is not well-studied.

Methods: A total of 32 female mice were, randomly divided into control group (CON, n=19) and depression group (DEP, n=13). The mice in the DEP group were subjected to 21 consecutive days of restraint stress, following depressive-like behaviors were assessment. The femurs were collected using Micro-Computed Tomography (μCT) and histochemical staining. In parallel, levels of serotonin-related proteins in the brain were measured using Western blot analysis, and sex hormone profiles were determined through liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS).

Results: The mice in the DEP group exhibited clear signs of depressive-like behaviors and an increase in serotonin transporter levels (t=-2.435, P< 0.05). In comparison to the CON mice, the DEP mice showed a decrease in bone mineral density (t =3.741, P< 0.05), bone surface area density (t =8.009, P<0.01), percent bone volume (t =4.293, P< 0.05), trabecular number (t =5.844, P<0.01), and connected density (t =11.000, P< 0.05). Additionally, there was an increase in trabecular separation (t =-7.436, P<0.01) in DEP mice. Furthermore, the DEP mice displayed a significant reduction in serum estrogen levels (t =4.340, P< 0.05) and changes in its metabolite (t =-3.325, P< 0.05), while the levels of androgens remained unchanged.

Conclusion: The restraint stress not only led to the development of depressive-like behaviors but also disrupted the estrogen metabolism pathway, resulting in damage to bone mass and microstructure in female mice. These findings suggest that stress-induced depression may pose a risk for bone loss in female mice by altering estrogen metabolism pathways.

Keywords: depression, bone mineral density, bone structure, estrogen, female

Introduction

Depression, a prevalent mental disorder, influences approximately 280 million individuals worldwide according to recent data from the World Health Organization (WHO), accounting for 3.8% of the world's population.[1] In China, the prevalence of depression is up to 54 million individuals,[2] with a lifetime prevalence of approximately 3.4%.[3] Moreover, depression also contributes to a significant disability burden, accounting for 37.3% of the disability-adjusted life years (DALYs) attributed to psychiatric illnesses.[4]

Recent etiology of depression includes neuroinflammation, gut microbiome's composition, genetic factors and alterations in brain circuits. For example, chronic inflammation is increasingly recognized as a key player in depression while anti-inflammatory treatments show promise in alleviating symptoms.[5–7] Studies also discovered that imbalances in gut bacteria may contribute to depression, and interventions like probiotics and dietary changes are being explored.[8] For genetics, investigations found that genes might influence vulnerability to depression and identifying specific genes and their interactions with environmental factors is crucial for personalized treatment.[9–11] Finally, advanced neuroimaging

Received: 14 December 2023
Accepted: 2 July 2024
Published: 18 July 2024

reveals alterations in brain circuits associated with mood regulation in depressed individuals. Understanding these changes may lead to targeted interventions like neuromodulation therapies.[12]

Depression is frequently comorbid with various conditions, such as osteoporosis.[4,6,13] Recent evidence has shown that depressed individuals tend to suffer from lower bone mass,[14,15] and displayed an increased susceptibility to osteoporosis and fractures compared to the general population.[16,17] Moreover, statistical analyses controlled for confounding factors including body height, weight, smoking status, and race have confirmed the correlation between depression and decreased bone mineral density (BMD).[18] Limited animal studies also support the hypothesis that depression results in bone loss and osteoporosis by measuring bone density and bone homeostasis.[19–21] However, there is a lack of evidence regarding the mechanisms that connect depression and disrupted bone homeostasis.

Epidemiological studies have demonstrated that women face a higher risk of developing both depression and osteoporosis compared to men.[22–24] Furthermore, women exhibit distinct clinical characteristics of depression, such as an earlier onset, longer duration, and higher frequency of recurrence compared to men.[25–27] Osteoporosis is also more prevalent in women, with a significant likelihood of experiencing an osteoporotic fracture during their lifetime.[28] Studies have shown that women generally have lower bone mineral densities (BMD) compared to men, and the majority of individuals with osteoporosis are women.[29] In addition to these epidemiological differences, sex disparities have been observed in the comorbidities, mortality rates, and response to drug therapy between men and women, with sex hormones playing a crucial role in these variations.[26,27,30–32] However, research on the cause-and-effect relationship between depression and bone loss is limited, and there is a lack of investigation into the sex differences in depression and osteoporosis due to the predominant focus on male animal studies.

In this study, our focus was to examine the connection between chronic stress-induced depression and bone microstructure in female mice. We aimed to investigate whether depression increases the risk of bone loss and determine the involvement of sex hormones in the disruption of bone homeostasis.

Methods and Materials

Animals

Total of 32 female C57Bl/6J mice, aged 8–12 weeks, were purchased from Vital River Co., Ltd and were group-housed under a controlled temperature, with chow and water available *ad libitum*. After one week of quarantine, the mice were randomly divided into two groups as depression group (n=13), and age-matched control group (n=19). After 21 days of CRS, Animals were tested for behavioral tests and sacrificed for tissue harvesting and further biological analyses. All studies were approved by the Ethical Committee for Animal Care and Use Committee of Capital Medical University. The experimental procedures were conducted following the National Institutes of Health Guide for the Care and Use of Laboratory Animals (IACUC: AEEI-2020-193).

CRS Paradigm

The CRS paradigm is an effective method for inducing depressive-like behaviors in rodents.[33–37] To enhance the effectiveness of depression induction, a modified version with a longer duration of 4–6 hours daily was utilized. The custom-designed devices (25 mm in diameter, 90 mm in length, with 4 small holes on both sides for heat dissipation and ventilation, from Cisco North Biotechnology Co., Ltd., CHN) allowed mice to extend their limbs and heads while limiting overall movement. In our study, the depression group was restraint in designated tubes for a total of 21 consecutive days while the control group remained in their cages undisturbed. After CRS or normal feeding, behaviour tests were performed on days 21, 23, 25 and 26, followed by tissue collection (see Figure 1).

Body Weight Measurement

All animals were weighed individually and documented in beakers. All animals were weighed individually and documented in beakers on the first day of weeks 1, 2, 3, and 4 of the CRS procedure. Therefore, the first measured body weight of the mice was day 0 before entering the CRS and the rest of the measured body weight was during the

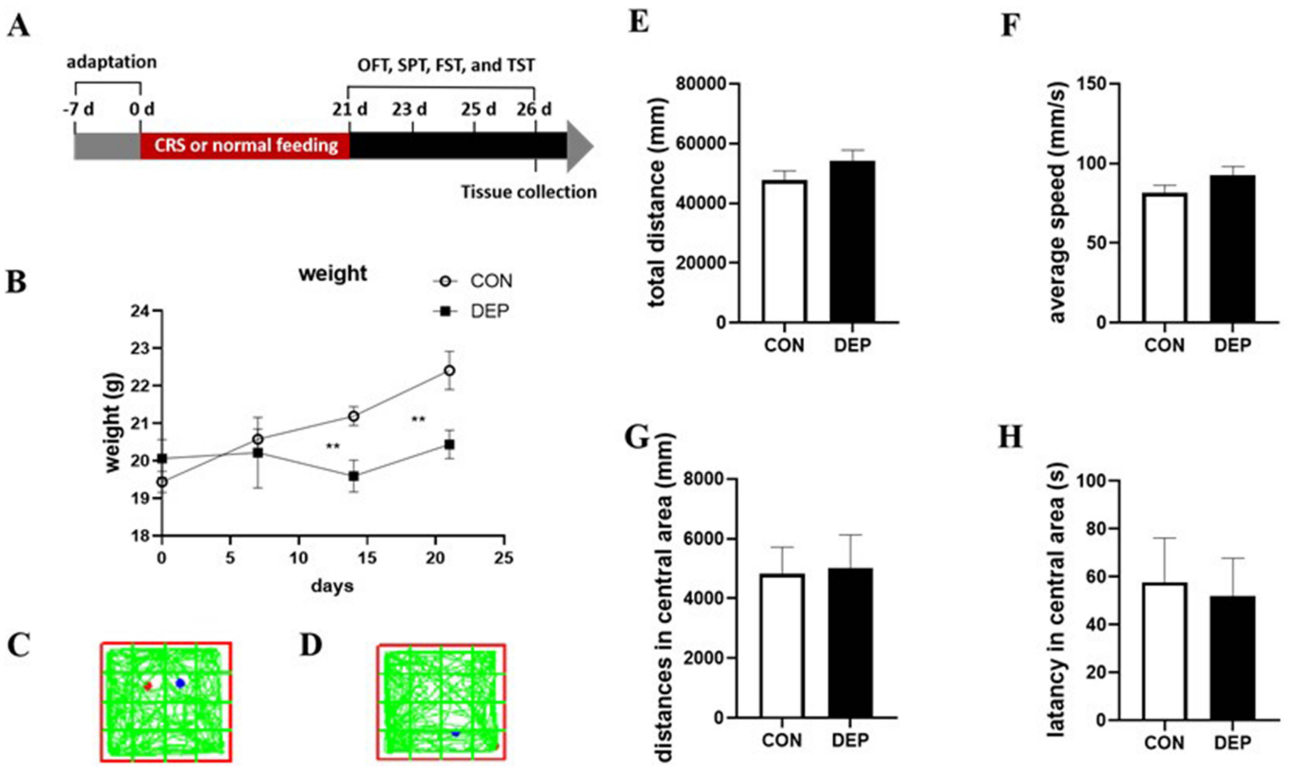

Figure 1 Stress induced weight loss but did not affect locomotion activity in female mice. Female C57Bl/6J mice were subjected to CRS-induced depression (DEP) or no treatment (CON). (**A**) Schema shows experimental schedule. (**B**) The body weight of our female mice during the period of restraint stress (CON, n=19 mice; DEP, n=13 mice). Representative motion trajectories of (**C**) CON mice and (**D**) DEP mice in OFT (CON, n=13 mice; DEP, n=11 mice). Animals were put into the OFT in the same site. The red and blue dots represented the monitored initial and terminal position, respectively. Locomotor parameters assessed in OFT including (**E**) total distance traveled, (**F**) average speed, (**G**) distances covered within the central region, and (**H**) retention time spent in the central region (CON, n=13 mice; DEP, n=11 mice). The results are represented as mean ± S.E.M.; $^{**}P$ value < 0.01.

CRS (days 7, 14, and 21). To minimize the potential impact of restraints on body weight measurements, each rodent was weighted at a consistent time point prior to inducing restraint stress.

Behavioral Tests

Open Field Test

The open field test (OFT) was performed following restraint procedure to assess the locomotor and anxiety-like conditions, as previously described.[38] All mice were placed in an open enclosure (50 cm ×50 cm × 40 cm, length × width × height) and their locomotion was recorded using the SuperMaze apparatus (XR-XZ301, XinRuan Co., Shanghai, CHN). The mice were given a 6-minute period to freely explore the unfamiliar plastic box while the surrounding environment remained quiet. Both central and overall activities were assessed using the software provided with the apparatus. To minimize potential olfactory interference from previous mice, the open enclosure was disinfected using 75% alcohol for each trial.

Sucrose Preference Test

The absence of hedonic response of animal model was evaluated by measuring the preference of sucrose in sucrose preference test (SPT), as demonstrated in prior investigations.[39–42] Mice were trained for 48 hours to drink two bottles of water to adapt, then after another 48-hour, they were given a bottle of water and 1% sucrose (w/v) to adapt to different solutions. On the day of testing, mice were deprived of solutions and food for 6 hours, and they then underwent a 48-hour preference test involving water and a 1% sucrose solution. To prevent location bias, the bottle positions were randomly alternated every 12 hours. Bottle weights were recorded both before and after the test. It is calculated using the ratio of (sucrose intake) to (sucrose plus water intake) as the percentage of sugar preference.

Forced Swim Test

The forced swim test (FST) was performed within the time range of 21:00–24:00, according to the previously reported methods.[37,43,44] A 26.5 cm high, 18 cm diameter clear transparent beaker containing 3500 mL water at 25±1°C was used to confine mice so that they neither escaped nor reached the bottom. The mice were gently introduced into the water for a duration of 6 minutes to record the immobility time. The cylinder was cleaned and refilled with fresh water following each test.

Tail Suspension Test

The tail suspension test (TST) for evaluating depressive-like behavior was described in previous references.[45,46] Mouse tails were carefully captured by their tails and suspended from the ground approximately 20 cm high. To prevent the mice from chasing their tails, an application of a small plastic cylinder was placed prior to suspension. The TST test was carried out for 6 minutes, with the final 4 minutes designated for subsequent analysis.

Determination of Samples

Micro-Computed Tomography

A 10% neutral formalin solution was used to fix the left femurs of all mice (Solaibao Biological Technology Co., Ltd., Beijing, CHN) for 48 hours at 4°C. Femurs were carefully secured in the tube to avoid any movement during scanning. Subsequently, micro-computed tomography (μCT) scanning was performed with a SkyScan 1172 (Bruker, Belgium) according to previous references.[47,48] In brief, a distinct area, constituting one-third of the distal growth plate and spanning from 100 to 500 slices across a length of 2.4 mm, was subjected to scanning. The image acquisition settings were as follows: tube voltage of 45 kV/165 μA, with a 0.5 mm filter. Images were acquired at an 8 μm resolution with a rotational step of 0.4°.

After scanning, the acquired data was subjected to tissue reconstruction and analysis using the NRecon software and CTAn software provided by the equipment manufacturer. Trabecular bone mass and microarchitecture, including bone mineral density (BMD), bone surface/volume ratio (BS/BV), percent bone volume (BV/TV), bone surface density (BS/TV), trabecular separation (Tb.Sp), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular degree of anisotropy (DA), connected density (Conn.D), bone pattern factor (Tb.Pf), and structure model index (SMI), were recorded.

Histological Examination

The left tibias, fixed as previously described, were decalcified using EDTA (pH=7.4) at 4°C for a minimum of 30 days until complete decalcification. Then, the tissues were processed according to the conventional paraffin method, based on a previous study.[49] The tissues were sectioned into 4μm sections and stained with hematoxylin and eosin (H&E) (Servicebio, Wuhan, CHN). The hematoxylin-eosin (H&E) staining was executed according to the protocol provided with the kit. Subsequently, the slides of femur were photographed by microscope DM2500 (Leica, Germany).

Protein Extraction and Western Blotting

Homogenization was performed on the brain tissues using RIPA lysate buffer on the brain tissues (Beyotime Biotech, Inc., CHN) containing protease inhibitor (Roche Pharmaceuticals, Inc., CH). Afterward, total protein concentrations were determined using the Bicinchoninic Acid Protein Assay Kit (Vazyme, CHN) as recommended by the manufacturer. According to previously described methods, Western blots were then performed.[50] In brief, equal amounts of protein lysates were loaded onto 10% sodium dodecyl sulfate polyacrylamide gels and subsequently transferred to nitrocellulose membranes (0.45 μm PVDF). The membranes were immunoblotted with the primary antibodies including anti-5-hydroxytryptamine 1A receptor (5-HT_{1A}R; 1:1000; Sigma-Aldrich, USA), anti-tryptophan hydroxylase 2 (TPH2; 1:1000; Sigma-Aldrich, USA), anti-serotonin transporter (5-HTT; 1:1000; proteintech, Inc., CHN), and GAPDH (1:50000; proteintech, Inc., CHN) at 4°C followed by appropriate HRP-secondary antibody for 90 min at room temperature. Chemiluminescence reagents (Pierce ECL Western Blotting Substrate; Thermo Fisher Scientific, USA) and multifunctional imaging system (Vilber Bio Imaging, FR) were used for detecting protein bends. ImageJ (http://imagej.net/ImageJ) was used to analyze the images.

Targeted Metabolomic Analysis

The LC-MS/MS method, a targeted liquid chromatography-mass spectrometry approach, was used for sex hormone metabolite profiling by a QTRAP 6500+ mass spectrometer (AB SCIEX, CA). The crude product was dissolved in methyl alcohol and analyzed by LC-MS, similar as described previously.[51,52] To separate the sex hormones and metabolites, we utilized a Waters ACQUITY UPLC HSS T3 C18 column. The mobile phase consisted of a 0.1% formic acid aqueous solution as mobile phase A and a 0.1% formic acid acetonitrile solution as mobile phase B. The elution was performed at a consistent flow rate of 0.35 mL/min. The eluted substances were ionized in positive mode using ESI. Standard curves were used to calculate substance concentrations.

Statistical Analysis

Statistical analysis was done by using SPSS statistical software (version 20.0; IBM, USA), and results are presented as mean ± standard error of mean (S.E.M). The results were visualized using GraphPad Prism 8.0 software downloaded from an official website (https://www.graphpad.com/scientific-software/prism/). Two-tailed unpaired Student's *t* test was used to assess the level of difference between two independent groups. A significance level of $P<0.05$ was accepted as statistically significant.

Results

CRS Induced a Less Body Weight Gain and No Effect on Locomotor Behavior

To examine whether stress-induced depression would lead to phenotypic shifts in mice, we designed a 21-days CRS stress experiment in female mice as shown in. In light of decreased body weight as fundamental clinical features of depression, we monitored the change of body weight weekly during the experiment process as long as the spontaneous and exploration locomotion activities in OFT. As shown in, Figure 1 the body weight of control animals increased steadily throughout while that in depressed mice increased slowly. At the first and second weeks, there was no significant difference in body weight between the control group and the depression group. However, depressed mice showed a significant lower body weight compared to their littermate controls in the third and fourth weeks (in the 3rd week, $t=3.452$, $P<0.01$; in the 4th week, $t=2.840$, $P<0.01$), respectively. In this study, depressed mice failed to induce any differences in OFT behaviors including the total distance traveled, speed of horizontal movement, the distance and time spent in the central region and latency in the central area compared to the control group ($P>0.05$) as shown in Figure 1. Our data suggested that the 21-days CRS induced a less body weight gain with age and had no significant effect on the locomotor behavior of female mice.

CRS Mice Developed Depressive-Like Behaviors and Promoted Serotonin Transporting

To investigate the depressogenic effect of CRS in mice, we conducted the FST, TST and SPT at the end of CRS procedure. As shown in Figure 2, the depression group showed significantly lower sucrose preference rates than control group ($t=2.941$, $P< 0.05$). In the FST and TST as shown in the Figure 2B and C, the depressed mice showed a longer immobility time compared to the control animals (FST, $t=-2.944$, $P< 0.05$; TST, $t=-2.348$, $P< 0.05$). Together, our behavioral data indicate that CRS can induce several depressive-like behaviors.

In light of the close relationship between serotonin (5-HT) and depression, we also examine the relative protein expression levels of $5\text{-HT}_{1A}\text{R}$, TPH2 and 5-HTT in the brain. Interestingly, an elevation of brain 5-HTT level was observed in the depressed mice ($t=-2.435$, $P< 0.05$) compared to controls while no significant difference in the relative expression levels of $5\text{-HT}_{1A}\text{R}$ protein ($t=-0.211$, $P> 0.05$) and TPH2 protein ($t=-1.339$, $P> 0.05$) were found between the two groups of animals.

CRS Induced Bone Mass Loss and Deterioration

In order to study the effect of depression on bone, we conducted histological examination using H&E staining and μCT analysis. The staining Results revealed that the chronic stress induced an structurally damaged in trabecular bone

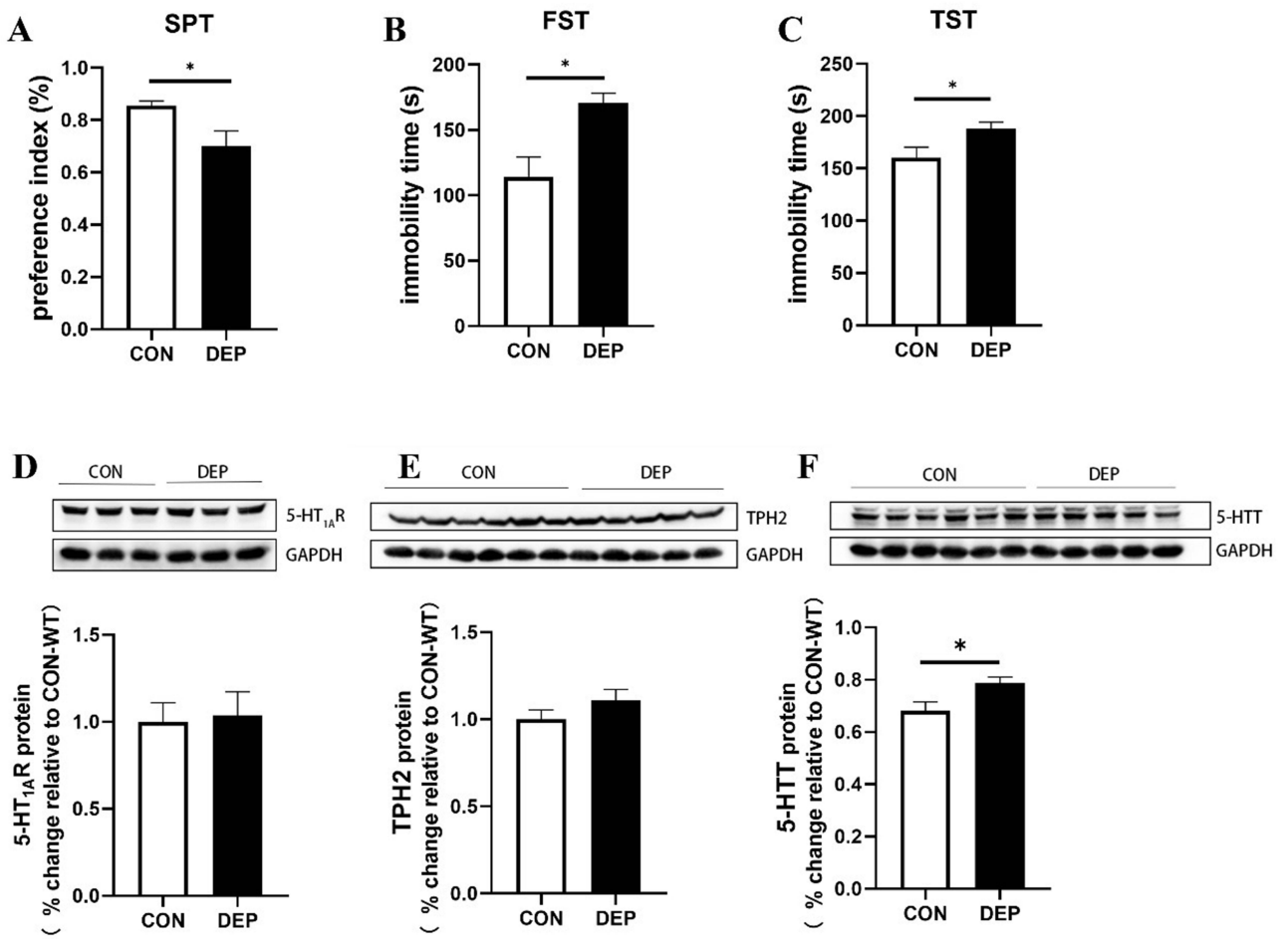

Figure 2 Stress induced depressive-like phenotypes and altered serotonin pathway in female mice. Results of behavioral tests including (**A**) sucrose preference ratio in the SPT (CON, n=18 mice; DEP, n=12 mice), (**B**) immobility time in the FST (CON, n=19 mice; DEP, n=13 mice), and (**C**) immobility time in the TST (CON, n=9 mice; DEP, n=9 mice). Results of Western blot detection and quantification including (**D**) 5-HT_{1A}R protein, (**E**) TPH2 protein, and (**F**) 5-HTT protein expression (CON, n=6 mice; DEP, n=5 mice). Results represent mean ± S.E.M.; *P< 0.05.

(Figure 3). Furthermore, we observed a decreasing trend in bone volume and an increasing trend in trabecular bone separation in the depressed mice.

To further confirm the stress-induced bone structure changes, we applied μCT analysis to quantify the microstructure changes of trabecular bone. As demonstrated in Figure 4, chronic stress led to a significant reduction in bone mass, in terms of BMD (t =3.741, P< 0.05), BV/TV (t =4.293, P< 0.05), and BS/TV (t =8.009, P<0.01), while BS/BV just showed a decreasing trend (t =0.853, P> 0.05). Meanwhile, the CRS mice displayed a notable deterioration in Conn.D (t =11.000, P < 0.05), but there were no significant differences in DA (t=0.116, P > 0.05), Tb.Pf (t =-0.210, P > 0.05), and SMI (t =-1.517, P > 0.05) between CRS and control animals, as shown in Figure 4. At the same time, a significant reduction in Tb.N (t =5.844, P<0.01) and increase in Tb.Sp (t =-7.436, P<0.01) was observed as a result of chronic stress, while there was no statistical difference in Tb.Th (t =-1.767, P > 0.05) between CRS group and control group. These findings suggest an abnormal trabecular structure and morphology in the bones of mice subjected to chronic stress.

CRS Altered Estrogen Turnover

To explore the mechanism how depression leads to the imbalance of bone homeostasis, we then further examined sex hormone metabolism pathways in mice serum. Chronic stress induced estrogen metabolism pathways altered, rather than the androgen (Figure 5). More specifically, depressed mice displayed a significant reduction of serum estrone, one of the types of estrogen (t =4.340, P< 0.05) compared with the control mice. The depressed mice also showed an elevated

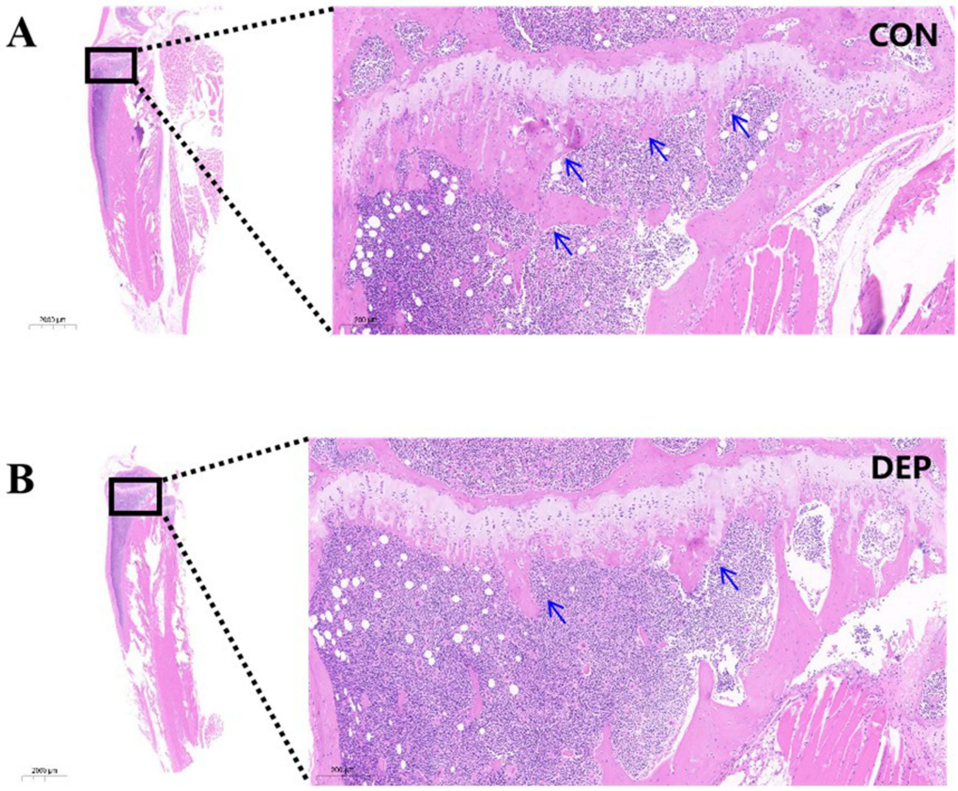

Figure 3 Histomorphology with H&E staining showed bone deterioration in stressed female mice. Representative histological images depicting H&E-stained tibia in (**A**) the control group and (**B**) the depression group (left panel, 2000 μm, scale bar; right panel, 200 μm, scale bar; n=4 mice per group). The blue arrow indicates the bone tissue.

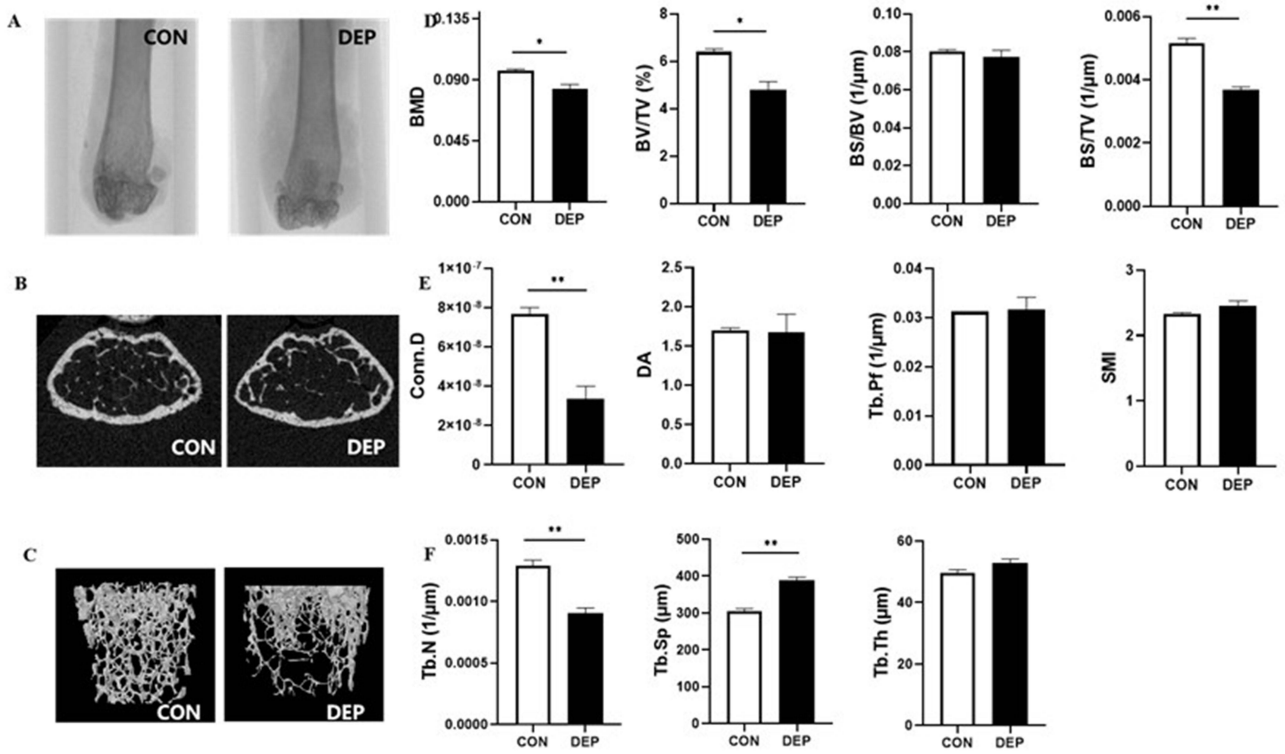

Figure 4 Quantitative μCT analysis showed bone deterioration and bone mass reduction in female depression mice. The images are representative of the metaphysis of a femur, shown in (**A**) sagittal sections, (**B**) coronal sections, and (**C**) 3D reconstruction. (**D**) The analysis of BMD, BV/TV, BS/BV, BS/TV. (**E**) Morphometric parameters of the trabecular bone in femora, including Conn.D, DA, Tb.Pf, SMI. (**F**) Bone structural parameters of the trabecular bone in femora, including Tb.N, Tb.Sp, Tb.Th (n=3 for each group).The results are presented as mean ± S.E.M.; **P*< 0.05, ***P*< 0.01.

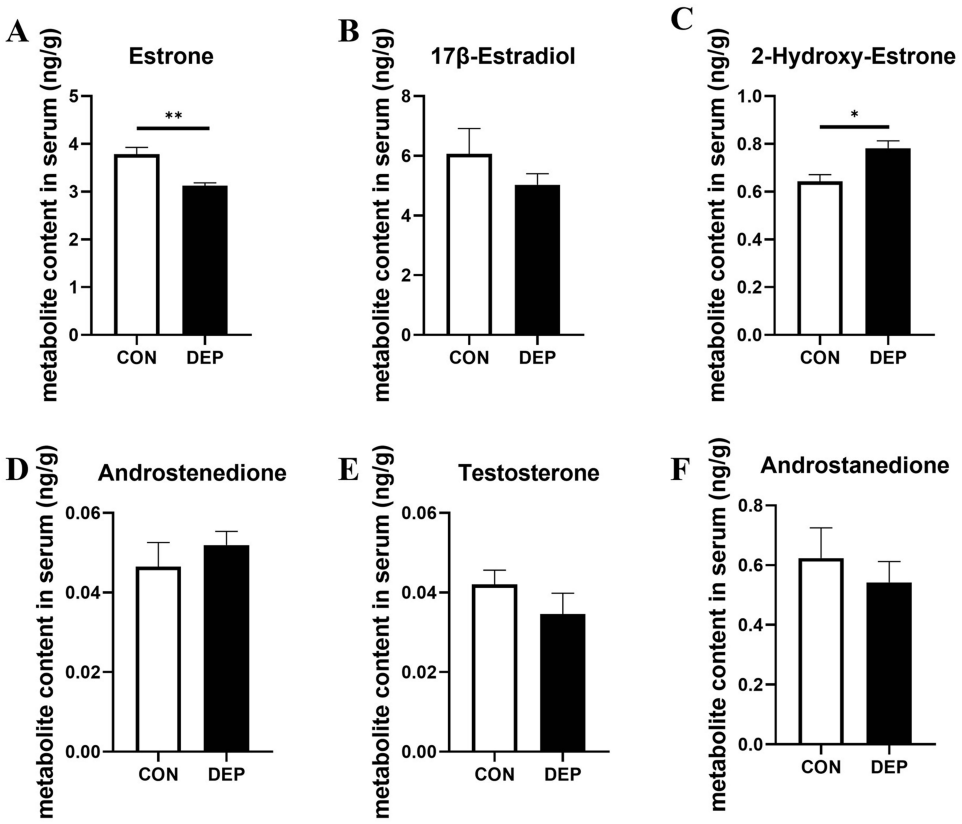

Figure 5 Depression induced changes in estrogen metabolites. Depression phenotype induces distinct changes in both (**A**) estrone and (**B**) 17β-estradiol, as well as (**C**) a metabolite, 2-hydroxy-estrone. (**D**) androstenedione and (**E**) testosterone, both male hormones, along with (**F**) a metabolite androstanedione (each group, n=6). The data are presented as mean ± S.E.M.; *P< 0.05, **P< 0.01.

serum level of estrogen metabolites 2-hydroxy-estrone (t =-3.325, P< 0.05) (Figure 5) with no changes found in 17β-estradiol levels (Figure 5). The CRS also failed to induce any significant changes in the levels of androgen as shown in Figure 5 including androstenedione (t =-0.777, P> 0.05), testosterone (t =1.169, P> 0.05), and androstanedione (t =0.223, P> 0.05). Our data suggested that stress-induced depression resulted in estrogen metabolism pathways may play an important role in bone homeostasis, not androgen.

Discussion

Although several clinical studies have highlighted bone loss in patients with depression,[14–17] the intricate mechanisms linking depressive states to disrupted bone homeostasis are not fully understood. Moreover, given that women are more prone to depression and osteopenia than men, our study sought to investigate the influence of depression on bone homeostasis in female mice. We utilized the CRS model to simulate depression in mice, as previously established.[33–37] Initially, we verified that CRS led to changes in body weight, a common symptom of depression.[53,54] While human studies indicate significant weight fluctuations in adults and minor changes in children with depression,[45] animal research shows a decrease in weight[55] or slower weight gain[56] in depressed subjects compared to their normal counterparts. We observed a notably slower increase in body weight in CRS-subjected mice compared to control animals, aligning our CRS model with the depression phenotype described in the literature.

Subsequently, we confirmed that female mice subjected to a 21-day CRS regimen exhibited several depressive-like behaviors, including increased immobility times in the FST and TST, indicative of despair,[45,57] reduced sugar water preference in the SPT as a measure of anhedonia,[39] and altered movement in the central region of the OFT for assessing anxiety-like behavior.[58] Post-21-day CRS, the mice demonstrated a decreased preference for sugar water and prolonged immobility in the FST, indicative of typical depression-like behaviors consistent with prior reports (Figure 2).[59–62]

However, no difference was observed in locomotor activity in the OFT between CRS and control groups (Figure 1). Our results align with some studies, such as Chiba et al, who reported no significant difference in OFT central region behavior and total distance between depression and control groups.[56] Other studies have shown that stress may not alter behavior in the central region but may decrease overall distance traveled,[55] particularly in female mice,[58] or reduce total distance, time spent in the central area, and climbing times in male depressed mice compared to sex-matched controls.[60,63] It is also noted that some research has shown changes in central distance without affecting total distance or central time in the OFT.[52,64] The consensus is that CRS is an effective model for depression-like behavior, with or without accompanying anxiety-like behavior, which could be due to variations in CRS protocols such as duration and frequency of restraint. It has also been suggested that genetic background may influence CRS outcomes, with mice of the C57Bl/6J strain showing less sensitivity to anxiety-like behaviors in OFT than in Elevated Plus Maze (EPM) tests.[65] Collectively, we verified depression-like behaviors without anxiety-like behavior in our depressed mice, and discrepancies observed in OFT may be attributed to sex[58] and species differences among experimental animals.[65]

Serotonin (5-HT) is known to play a significant role in depression, particularly in females who are at a higher risk for both depression and bone-related issues.[66–69] To explore the impact of depression on bone health, we first assessed the expression levels of serotonin transporter (5-HTT), receptor (5-HT_{1A}R), and the key enzyme for 5-HT synthesis (TPH2) in the whole brain of mice. We found an increase in 5-HTT expression following CRS without changes in 5-HT_{1A}R and TPH2 protein levels (Figure 2). Our data suggest that stress-induced depression in female mice enhances 5-HT reuptake through upregulation of 5-HTT expression in the brain, which aligns with findings from human studies.[70] However, we observed no impact on 5-HT synthesis or on the receptor 5-HT_{1A}R, while literature indicates that the function of 5-HT_{1A} R varies among depressed patients and animal models,[71] eliciting different responses across various brain regions upon stress exposure.[72] Conversely, despite TPH2's primary role in brain 5-HT synthesis, its expression appears remarkably resilient to stress.[73,74]

To assess depression's effect on bone health, we evaluated bone mass and microstructure using bone imaging analysis and histological staining (Figures 3 and 4). We noted significant decreases in bone mineral density (BMD), bone volume fraction (BV/TV), and bone surface fraction (BS/TV) in depressed mice compared to controls (Figure 4), suggesting that CRS-induced depression can precipitate bone loss in female mice—a finding supported by preclinical studies in male models[75,76] and numerous clinical investigations.[14,15] Additionally, micro-CT scanning revealed increased trabecular separation (Tb.Sp) and reduced trabecular number (Tb.N) in depressed mice versus controls (Figure 4), with no significant differences found in trabecular thickness (Tb.Th), degree of anisotropy (DA), connectivity density (Conn. D), trabecular pattern factor (Tb.Pf), or structure model index (SMI). These results corroborate previous research demonstrating bone remodeling under chronic stress, including increased Tb.Sp and decreased Tb.N in male models.[20,21] While fewer studies have been conducted on female subjects, one report noted no changes in trabecular characteristics following chronic mild stress exposure in female mice.[19] In our analysis of trabecular morphology parameters, only Conn.D was reduced, aligning with earlier findings.[19,20] Multiple lines of evidence suggest that conditions like depression, post-traumatic stress disorder (PTSD), and social isolation can heighten osteoporosis risk in both humans and animals,[77,78] with the effects of stress- or depression-related bone loss being sex-dependent. For instance, a recent study indicated that one month of social isolation significantly decreased trabecular and cortical bone parameters in male but not female mice, including BV/TV, BMD, Tb.N, and Tb.Th.[79]

To further elucidate the role of sex hormones on bone health in female mice, we analyzed estrogen metabolites. Notably, CRS-induced skeletal damage in female mice may be associated with alterations in estrogen metabolism pathways as detected by LC-MS/MS. Since both depression and osteoporosis are linked with estrogen levels, we measured serum concentrations of 17β-estradiol, estriol, and estrone. Our findings showed no change in 17β-estradiol but a decrease in estrone levels following CRS (Figure 5). Estrone, one of the three major endogenous estrogens along with 17β-estradiol and estriol,[80] has a close relationship with estradiol levels, and lower estrone levels may also increase osteoporosis risk.[81] Although estrone's direct regulatory effect on bone health remains unclear, it may indirectly influence bone health through its role in hormonal balance and estrogen production.[82] Our study's observation of reduced estrone levels suggests that CRS-induced depression and subsequent bone loss might be related to altered estrogen production, potentially regulating bone health indirectly.

Our study investigated the negative association between depression-like behaviors and bone mass and bone micro-architecture in rodents. We also found that depressed rodents had a reduction in levels of estradiol and estrone while 2-hydroxy-estrone levels were elevated, suggesting that abnormalities in the estrogen metabolism may play roles in the negative association between depression and bone mass. Our finding not only provides evidence on estrogen involvement in depression and bone health, but also indicates an important target for women who abnormal estrogen balance for depression and osteoporosis prevention. In the future, we will further investigate the molecular pathways of estrogen system in depression-induced changes in bone. Secondly, using more advanced technologies, we will further identify the tissue- and cell-specific targets for estrogen-related genes or proteins that serve as potential biomarkers for depression and bone loss early diagnosis and prevention.

Conclusion

In Conclusion, our research demonstrates that restraint stress induces depressive-like behaviors and disrupts serotonin reuptake and estrogen production, leading to compromised bone mass and microstructure integrity in female mice. These insights offer a new perspective on pathophysiological mechanisms behind bone density abnormalities associated with depression and underscore the importance of integrative research that considers sex as a biological variable when examining the relationship between neuropsychiatric disorders and skeletal health. Ultimately, our study enriches the understanding of stress-induced depression's effects on bone microstructure beyond BMD assessments. Considering females' increased vulnerability to both depression and osteoporosis, our findings also underscore the potential role of estrogen and its metabolites in skeletal deterioration associated with depression, aiming to improve strategies for preserving women's bone health.

Acknowledgment

Guoqiang Yan from Beijing University affiliated with Jishuitan Hospital, China provided support for the detection of bone morphology using μCT.

Funding

The research was supported by Sci-Tech Innovation 2030 - Major Project of Brain science and brain-inspired intelligence technology: 2021ZD0200600.

Disclosure

The authors report no conflicts of interest in this work.

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