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Treadmill exercise prevents reduction of bone mineral density after myocardial infarction in apolipoprotein E-deficient mice

Masanori Kanazawa, Yasuharu Matsumoto, Kikuyo Takahashi, Hideaki Suzuki, Hironori Uzuka, Kensuke Nishimiya and Hiroaki Shimokawa

Abstract

Aims: Recent clinical studies demonstrated the association between myocardial infarction (MI) and osteoporotic fractures. We examined whether MI causes bone loss and the effects of exercise training on bone in mice after MI.

Methods: We created a MI model in 16-week-old male apolipoprotein E-deficient mice ($n = 42$), which were randomly assigned to exercise group (MI-Ex) and sedentary group (MI-Sed). We also performed sham operations in other mice ($n = 10$). Treadmill exercise training was performed from one week after operation to eight weeks. At eight weeks, the bone parameters of the femur were measured by quantitative computed tomography, followed by histological analysis ($n = 10$–17).

Results: Bone mineral density (BMD) of the femur was significantly decreased in the MI-Sed group as compared with the sham group ($P < 0.001$), whereas the BMD was significantly increased in the MI-Ex group as compared with the MI-Sed group ($P < 0.05$). In histological analysis, Rho-associated coiled-coil kinase 2 and tartrate-resistant acid phosphate positive (bone resorptive) area in distal femur were significantly increased in the MI-Sed group as compared with the sham group ($P < 0.05$), whereas those parameters were significantly decreased in the MI-Ex group as compared with the MI-Sed group ($P < 0.05$). In contrast, alkaline phosphatase (ALP)-positive (bone-forming) area was significantly decreased in the MI-Sed group as compared with the sham group ($P < 0.05$), whereas ALP-positive area was significantly increased in the MI-Ex group as compared with the MI-Sed group ($P < 0.05$).

Conclusions: The present study demonstrates that MI reduces BMD and treadmill exercise training prevents the reduction of BMD in apolipoprotein E-deficient mice.

Keywords

Exercise, myocardial infarction, bone, osteoporosis, mice

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Introduction

Osteoporosis is characterized by low bone mass with a resultant bone fragility and, thus, susceptibility to fracture, especially at the proximal femur (hip) and the vertebrae.[1] Importantly, osteoporotic fracture is one of the most common causes of disability and demand of medical care costs in the world with aging population.[2] Indeed, hip fracture is the most serious result of osteoporosis as it 1) reduces the quality of life due to severe pain and disability; 2) forces the patients to spend time in a rehabilitation facility; and 3) even causes high mortality rates.[1] The estimated number of hip fractures worldwide will rise approximately four-fold from 1990 to 2050.[3] Thus, osteoporosis has been recognized as one of the most important public health issues.

Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan

Corresponding author:
Yasuharu Matsumoto, Department of Cardiovascular Medicine Tohoku University Graduate School of Medicine Seiryo-machi, Aoba-ku Sendai 980-8574, Japan.
Email: matsumoto@cardio.med.tohoku.ac.jp

Similarly, chronic heart failure (HF) is also a leading cause of hospitalizations and death in rapidly aging society worldwide.[4] The number of patients with cardiovascular risk factors, such as dyslipidemia, hypertension and diabetes mellitus, has been increasing with a resultant increase in the incidence of myocardial infarction (MI).[5] Importantly, recent studies indicated the relation between cardiovascular diseases (CVDs) and risk of subsequent hip fractures.[6,7] Furthermore, recent large cohort studies have demonstrated that the association between the history of MI and osteoporotic fracture has become more evident in the past decades.[8]

Bone mineral density (BMD) is the most important factor regulating bone strength, accounting for approximately 70% of bone strength.[9] Bone remodeling is a temporally regulated process with the balance between resorption and formation of bone tissue.[1] Notably, osteoclasts activation increases bone resorption activity and tips the balance in favor of resorption of bone, resulting in low BMD and osteoporosis.[1] Although exercise training has been shown to reduce CVD,[10] there is no experimental model showing the link between MI and bone loss.

In the present study, we thus aimed to examine whether MI causes structural loss of bone in a MI model of apolipoprotein E-deficient (ApoE-KO) mice. And if so, we also aimed to examine whether exercise training after MI is able to prevent the reduction of BMD after MI and its potential mechanism.

Methods

In the present study, we created an experimental MI model in 16-week-old male apolipoprotein E-deficient mice ($n = 42$), which were randomly assigned to an exercise group (MI-Ex; $n = 18$) and a sedentary group (MI-Sed; $n = 24$) (Figure 1). We also performed sham operations in other mice ($n = 10$). At eight weeks, the bone parameters of the femur were measured by quantitative computed tomography (CT), followed by

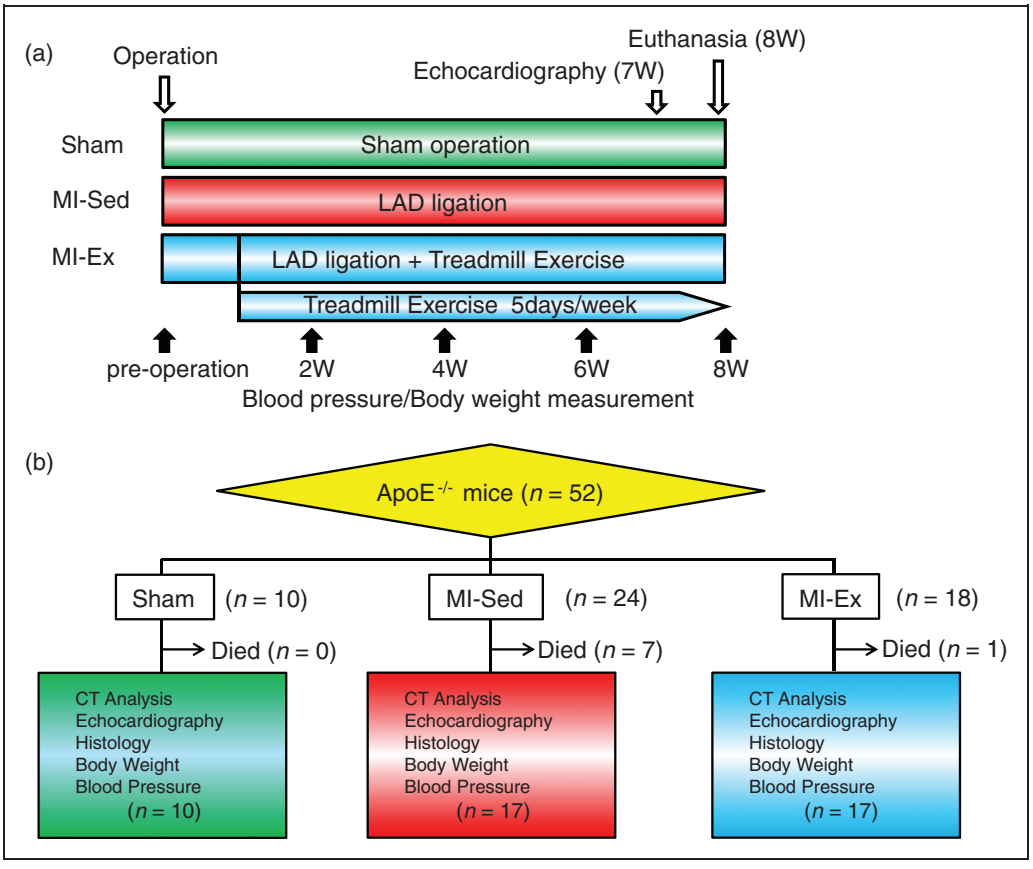

Figure 1. Experimental study design. Three treatment groups were created in the present study: sham group (sham), acute myocardial infarction group treated with LAD coronary artery ligation at 16 weeks old without exercise training by treadmill (MI-Sed), and acute myocardial infarction group with exercise training by treadmill (MI-Ex). The time points (a) and measurements (b) of the study are highlighted.

LAD: left anterior descending coronary artery; MI: myocardial infarction.

histological analysis ($n = 10$–17). Detailed methods are available in the online supplementary material.

Results

Blood pressure and body weight

Before the ligation of the left anterior descending (LAD) coronary artery, there were no significant differences in body weight or blood pressure among the sham, the MI-Sed, and MI-Ex groups (see online table). Every two weeks after MI, there were no significant differences in each parameter among the 3 groups (online table).

Blood chemistry

Serum levels of calcium and phosphorus were all comparable between the three groups (online table).

Echocardiography and histology

At seven weeks after MI, echocardiography was performed. The motion of the anterior wall in the MI group appeared akinetic. In contrast, the sham group showed no asynergy (online Figure 1(a)). Left ventricular ejection fraction (LVEF) was significantly decreased in the MI group as compared with the sham group (MI-Sed $26.4 \pm 3.4\%$, MI-Ex $25.8 \pm 3.4\%$ versus sham $61.4 \pm 4.0\%$; $P < 0.001$ each) (online Figure 1(b)). Left ventricular fractional shortening (LVFS) was also significantly decreased in the MI group as compared with the sham group (MI-Sed $12.7 \pm 1.9\%$, MI-Ex $12.4 \pm 1.9\%$ versus sham $32.5 \pm 0.9\%$; $P < 0.001$ each) (online Figure 1(b)). Left ventricular diastolic dimension was significantly increased in the MI-Sed and MI-Ex group as compared with the sham group (MI-Sed 5.4 ± 0.2, MI-Ex 5.4 ± 0.2 versus sham 3.9 ± 0.1 mm; $P < 0.001$ each) (online Figure 1(b)). Histological analysis of the heart showed that left ventricular (LV) infarct size (% of LV) was comparable between the MI-Sed group ($36 \pm 3\%$) and the MI-Ex group ($37 \pm 4\%$), whereas no LV infarct was noted in the sham group (online Figure 2).

CT analysis of the bone

Quantitative CT analysis demonstrated that the total BMD of the femur was significantly lower in the MI-Sed group as compared with the sham group, whereas the total BMD was significantly increased in the MI-Ex group as compared with the MI-Sed group (MI-Sed 598.1 ± 3.8 versus sham 626.1 ± 4.8 mg/cm^3, $P < 0.05$, versus MI-Ex 616.1 ± 5.3, $P < 0.05$)

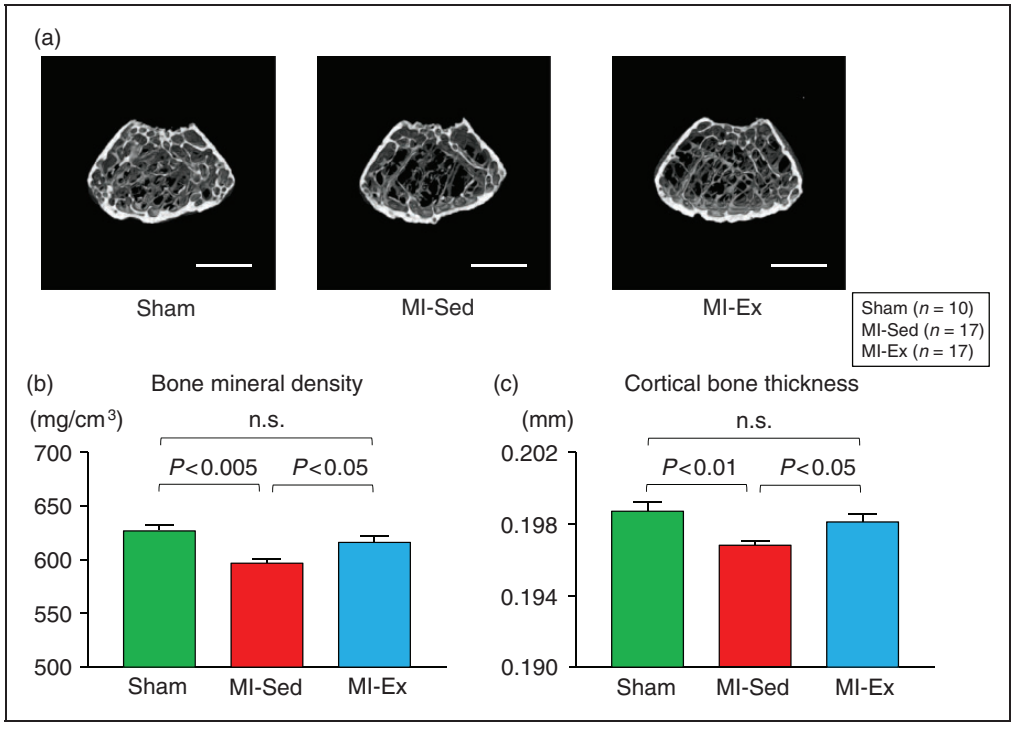

Figure 2. Analysis of bone parameters by CT. Representative microstructural images of metaphysis by micro-CT were shown in the sham group, MI-Sed group and MI-Ex group (a). Scale bars = 1 mm. Total bone mineral density (b) and cortical bone thickness (c) were measured. Results are expressed as mean ± standard error of the mean (SEM).

(Figure 2(a) and (b)). The cortical bone thickness was also significantly decreased in the MI-Sed group as compared with the sham group and MI-Ex group (MI-Sed 0.197 ± 0.0003 versus sham 0.199 ± 0.0005 mm, $P < 0.001$, versus MI-Ex 0.198 ± 0.0004 mm, $P < 0.05$) (Figure 2(c)). The trabecular density was significantly low in the MI-Sed group as compared with the sham group and MI-Ex group (MI-Sed 350.3 ± 4.3 versus sham 378.2 ± 5.3 mg/cm^3, $P < 0.005$, versus MI-Ex 369.6 ± 5.6 mg/cm^3, $P < 0.05$).

Bone immunohistochemistry

Semiquantitative analysis of immunohistochemistry demonstrated that the number of tartrate-resistant acid phosphate (TRAP)-positive areas in metaphysis was also significantly increased in the MI-Sed group as compared with the sham group, whereas the number of TRAP-positive areas was significantly decreased in the MI-Ex group as compared with the MI-Sed group (Figure 3). In contrast, the number of alkaline phosphatase (ALP)-positive areas was significantly decreased in the MI-Sed group as compared with the sham group, whereas the number of ALP-positive areas was significantly increased in the MI-Ex group as compared with the MI-Sed group (Figure 4). The number of Rho-associated coiled-coil kinase 2 (ROCK2)-positive areas was significantly increased in the MI-Sed group as compared with the sham group, whereas the number of ROCK2-positive areas was significantly decreased in the MI-Ex group as compared with the MI-Sed group (Figure 5).

Discussion

The major findings of the present study were that 1) post-infarction HF significantly reduced BMD and cortical bone thickness and increased ROCK2 expression in the femur of ApoE-KO mice; 2) exercise training prevented the reduction of BMD and cortical bone thickness after MI; 3) exercise training reduced bone resorption activity of osteoclast; 4) exercise training accelerated bone formation activity; and 5) exercise training reduced ROCK2 expression in the bone tissue. To the best of our knowledge, this is the first study that demonstrates that post-infarction HF causes reduction in BMD, and exercise training prevents the reduction of BMD in mice with post-infarction HF.

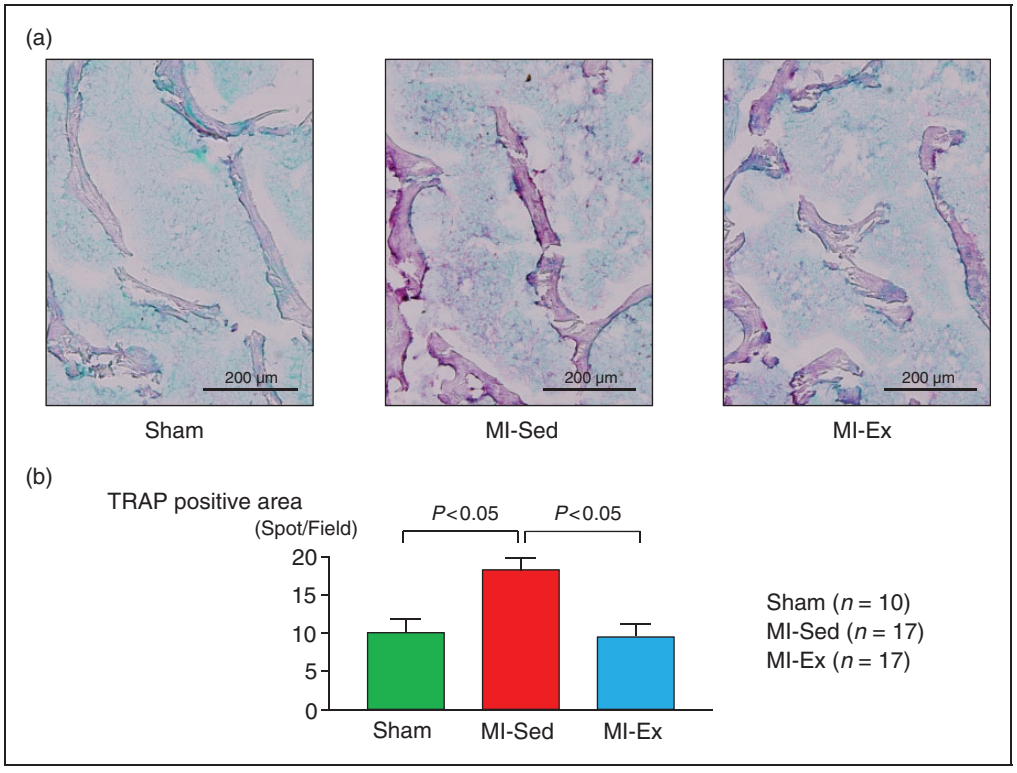

Figure 3. Histological analysis for TRAP staining of bone tissue. Representative histology for TRAP staining for the bone resorption activity of the metaphyseal area of the femur was demonstrated in the sham group, the MI-Sed group and the MI-Ex group (a). The semi-quantitative analysis of TRAP-positive areas of metaphysis was examined (b). Scale bars = 200 μm. Results are expressed as mean ± SEM.

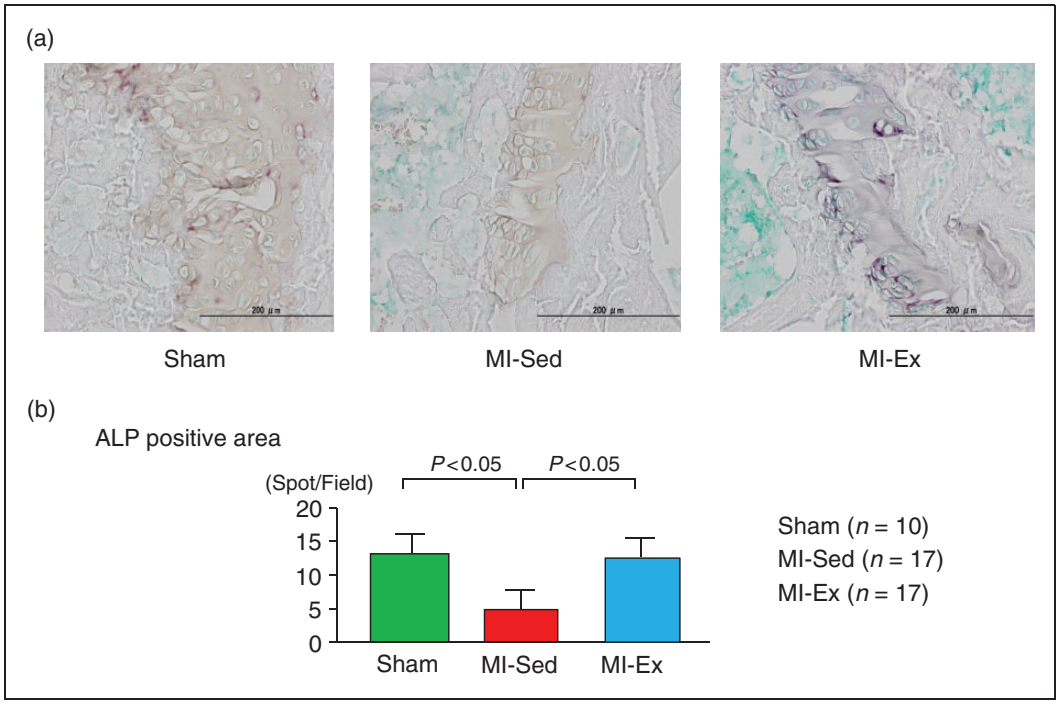

Figure 4. Histological analysis for ALP staining of bone tissue. Representative immunohistochemistry for ALP staining of the femur for bone morph genic activity was demonstrated in the sham group, the MI-Sed group and the MI-Ex group (a). The semi-quantitative analysis of ALP-positive areas of metaphysis was examined (b). Scale bars = 200 μm. Results are expressed as mean ± SEM.

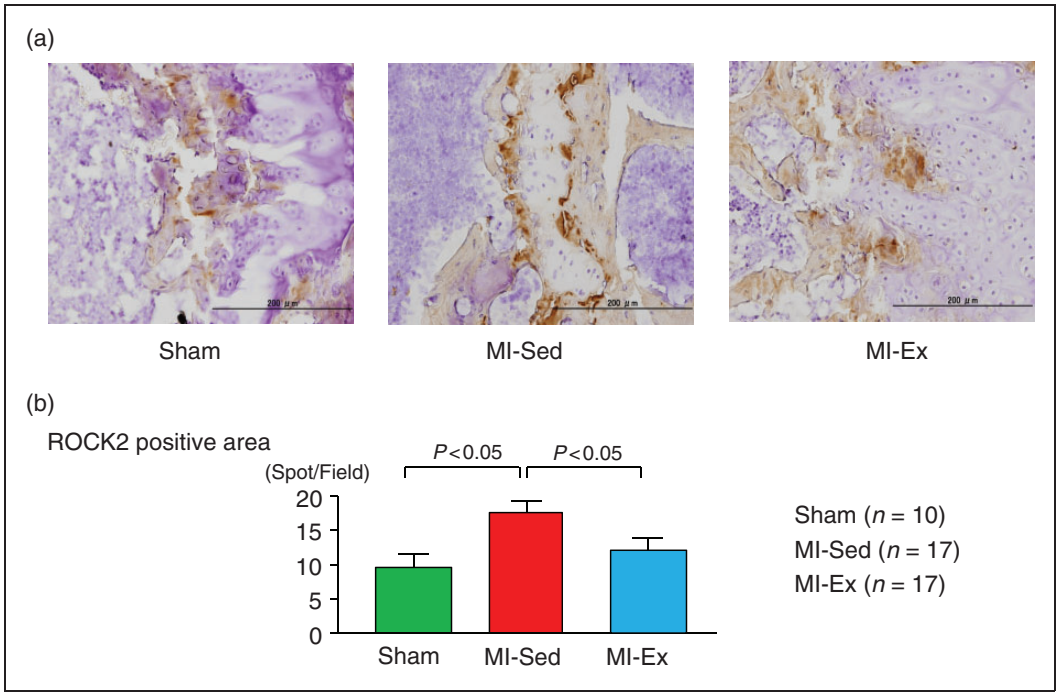

Figure 5. Histological analysis for ROCK2 staining of bone tissue. Representative histology for ROCK2 staining of femur bone tissue focused on the trabecular was demonstrated in the sham group, the MI-Sed group and the MI-Ex group (a). The semi-quantitative analysis of ROCK2-positive areas of metaphysis was examined (b). Scale bars = 200 μm. Results are expressed as mean ± SEM.

MI decreases bone mass and strength

Accumulating evidence links atherosclerotic vascular factors to osteoporotic fractures.[11–13] The previous large cohort studies showed that patients with chronic HF have lower BMD.[14] However, it remains unknown whether CVD (e.g. MI) directly causes bone tissue loss. The present study has demonstrated that MI causes a loss of BMD and cortical bone thickness in mice in vivo.

It has been recently demonstrated that cortical bone thickness could be an important factor regulating bone strength.[15] Osteoporosis is defined by the World Health Organization as a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a resultant increase in fracture risk.[16] Osteoporosis is the condition with weakened bone strength, and bone fractures would occur easily by a slight hit or small injury. Importantly, the present study showed that MI reduces not only BMD but also cortical bone thickness. These results indicate that MI decreased bone strength and enhanced bone fragility. Thus, the present experimental model may be close to the clinical conditions in patients with osteoporosis.

MI changes bone resorption and morphogenic activity

In the present study, the histological examination showed that the number of TRAP-positive spots (bone resorption areas) was significantly increased and ALP-positive areas were decreased in the MI-Sed group, indicating MI might increase osteoclast and decrease osteoblast activity in the bone after MI. These results suggest that the bone metabolism was driven to high turnover state in favor of bone resorption over bone formation after MI.

Potential common pathway underlying post-infarction bone loss

An experimental MI model by ligating LAD coronary artery in mice is one of the most commonly used models of HF.[17] There are several epidemiological studies indicating the association between HF and osteoporosis.[18] In addition, there are some additional confounding factors as many HF patients are elderly people and aging is a major risk for osteoporosis.[19] However, the experimental animal model of MI can exclude these confounding factors and help us identify potential common pathophysiological mechanisms linking HF and osteoporosis.

The renin-angiotensin system (RAS) is an essential regulatory component of the cardiovascular system and bone metabolism.[20–24] We and others have recently demonstrated that RAS is involved in the pathogenesis of bone turnover.[22–24] Although we were unable to show RAS activation in the present study, we previously demonstrated that activation of the RAS is, indeed, noted in mice with spontaneous MI model.[25]

An experimental study has shown that angiotensin II accelerates bone resorption by activating osteoclasts via the receptor activator of the nuclear factor-kappa B ligand (RANKL) pathway, and this effect was completely blocked by an angiotensin II type 1 receptor blocker.[23] Furthermore, it has been recently demonstrated that in an experimental MI model in mice, the plasma levels of RANKL and the number of TRAP-positive cells were markedly increased,[24] indicating that the activation of bone mineral metabolism promotes catabolic bone remodeling. Based on these studies, combined with the present findings, the RAS–RANKL bone resorption pathway may be one of the underlying mechanisms linking both disorders.

We have repeatedly demonstrated that the Rho-kinase pathway is as important in the pathogenesis of CVD and that Rho-kinase is also involved in the bone metabolism.[26–28] We also have demonstrated that angiotensin II induces ROCK activation,[29] whereas the Rho-kinase inhibitor fasudil suppresses post MI cardiac remodeling.[30] In the present study, we were able to demonstrate for the first time that elevated levels of ROCK2 expression in the bone is noted in mice with MI. Notably, fasudil, an inhibitor of Rho-kinase, augments osteoblastic differentiation in stromal cell lines.[28] Importantly, exercise training and fasudil reduce Rho-kinase activity.[26,31] Taken together, exercise training after MI is beneficial for bone resorption and morphogenetic activity toward the balance to increase BMD in mice with MI.

Study limitations

Several limitations should be mentioned for the present study. First, although we were able to demonstrate that our exercise protocol influences BMD in the present study, we did not have proof of a profit from the exercise training intervention such as a maximal exercise test. Taking into account the effects of exercise, particularly on bone metabolism as previously reported,[32–34] we performed an exercise protocol with a steeper uphill angle, shorter duration and more frequent interval, compared with endurance exercise training.[35] Indeed, vertical climbing with short duration time,[32] jump exercise[33] and hyper gravity environment[34] increase BMD in rodents. Second, the bone quality that accounts for ~30% of bone strength was not examined in the present study.[9] Although some factors such as pentosidine or homocysteine can be utilized as markers of bone quality in humans,[36,37] a reliable marker has yet to be established in mice. Third, we did not perform

echocardiography before starting protocol with exercise training. However, histological LV myocardial infarct size was comparable between the two groups. Fourth, in order to eliminate the potential effects of hormonal changes on bone metabolism during the menstruation period and to consider osteoporotic risks shared with atherosclerosis,[7,38,39] we used male apolipoprotein E-deficient mice in the present study.

Conclusion

The present study demonstrates for the first time that post-infarction HF directly causes structural bone loss of the femur in ApoE-KO, which is ameliorated by treadmill exercise training, suggesting importance of close attention to the bone loss and potential importance of cardiac rehabilitation in terms of exercise training in patients with MI.

Author contribution

YM and HS were responsible for the conceptualization of the design. MK and YM conducted the data analysis. MK, YM and HS drafted the manuscript. MK, YM, KT, HS, HU and KN contributed to acquisition or interpretation of the data. All authors gave final approval and agreed to be accountable for all aspects of work to ensure integrity and accuracy.

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Declaration of conflicting interests

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