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Moderate Joint Loading Reduces Degenerative Actions of Matrix Metalloproteinases in the Articular Cartilage of Mouse Ulnae

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Abstract

Joint loading is a recently developed loading modality, which can enhance bone formation and accelerate healing of bone fracture. Since mechanical stimulation alters expression of matrix metalloproteinases (MMPs) in chondrocytes, a question addressed herein was, does joint loading alter actions of MMPs in the articular cartilage? We hypothesized that expression and activity of MMPs are regulated in a load–intensity-dependent manner and that moderate load scan downregulates MMPs. To test this hypothesis, a mouse elbow-loading model was employed. In the articular cartilage of an ulna, the mRNA levels of a group of MMPs as well as their degenerative activities were determined. The result revealed that elbow loading altered the expression and activities of MMPs depending on its loading intensity. Collectively, the data in this study indicate that 0.2 and 0.5 N joint loading significantly reduced the expression of multiple MMPs, that is, MMP-1, MMP-3, MMP-8, and MMP-13, and overall activities of collagenases or gelatinases in articular cartilage, while higher loads increased the expression and activity of MMP-1 and MMP-13. Furthermore, moderate loads at 1 N elevated the mRNA level of CBP/p300-interacting transactivator with ED-rich tail 2 (CITED2), but higher loads at 4 N did not induce a detectable amount of CITED2 mRNA. Since CITED2 is known to mediate the downregulation of MMP-1 and MMP-13, the result indicates that joint loading at moderate intensity reduces MMP activities through potential induction of CITED2. MMPs such as MMP-1 and MMP-13 are predominant collagenases in the pathology of osteoarthritis. Therefore, joint loading could offer an interventional regimen for maintenance of joint tissues.

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Keywords

elbow loading; ulna; articular cartilage; matrix metalloproteinase; CITED2

INTRODUCTION

Mechanical loading affects bone remodeling in an intensity-dependent manner. Moderate exercise increases bone mineral density and prevents bone loss [1]. A lack of mechanical stimulation, as seen in bedridden patients or astronauts during spaceflight, leads to rapid loss of bone mass [2], while mechanical overloading causes bone fractures [3]. As a novel mechanical interventional regimen, we previously developed a joint loading modality [4]. This modality applies lateral loads to a synovial joint such as the elbow, ankle, and knee and stimulates bone formation throughout the length of long bones [5]. Although loads are directly given to joint tissues, the loading effects on the articular cartilage, one of the major constituents of the joint tissues, have not been investigated.

Articular cartilage in a synovial joint is composed of chondrocytes embedded in an extracellular matrix (ECM), which is rich in type II collagen and proteoglycans [6]. It functions as a nearly frictionless mechanical bearing that transfers dynamic loads on underlying bone. Like bone, cartilage is known to respond to mechanical loading in an intensity-dependent manner. Reduced loads in bedridden patients or astronauts generate degenerative responses in articular cartilage [7]. Acute or chronic high-intensity loads, as seen in athletes participating in high-impact sports, often predispose the joint to osteoarthritis [8]. In this study, we addressed a set of questions: Does joint loading alter the expression and activities of matrix metalloproteinases (MMPs) in the articular cartilage? If yes, what loading condition reduces the expression and proteolytic enzyme activities of MMPs? We hypothesized that the effects of joint loading are dependent on loading intensities and that moderate loads can suppress the expression and activities of predominant collagenases in the articular cartilage, that is, MMP-1 and MMP-13 [9].

To test the above hypotheses, we employed elbow loading with loading intensities ranging from 0.2 to 4 N (peak to peak). It is reported that loads at 0.5 N on the elbow were capable of stimulating bone formation throughout the ulna including the proximal and distal diaphyses [10]. In this study, we first determined the strain induced by three loading intensities (0.2, 0.5, and 2 N) in the metaphysis and diaphysis of the ulna. Prior to testing dependence of MMP expression and activities on loading intensities, this measurement examined potential dependence of induced strains on loading intensities in the ulna.

Using three loading intensities, we determined the mRNA expression levels of five MMPs (MMP-1, MMP-3, MMP-8, MMP-13, and MMP-14) and two tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2). The mRNA levels of osteocalcin and CBP/p300-interacting transactivator with ED-rich tail 2 (CITED2) were also determined. Osteocalcin is an anabolic bone marker [11], which served to validate the efficacy of elbow loading in bone formation in the ulna. CITED2 is a transcription regulator that is reported to suppress the expression of MMP-1 and MMP-13 [12]. To evaluate various degenerative activities of

MMPs, we determined total activities of collagenases and total activities of gelatinases, as well as MMP-1- and MMP-13-selective activities.

MATERIALS AND METHODS

Animals

Experimental procedures were approved by the Indiana University Animal Care and Use Committee and were in compliance with the Guiding Principles in the Care and Use of Animals endorsed by the American Physiological Society. Twenty-four C57/BL/6 female mice, ~12 weeks of age (Harlan Sprague-Dawley), were used. Four to five mice were housed per cage and they were fed with mouse chow and water ad libitum. The animals were allowed to acclimate for 1 week before the experiment.

Elbow Loading

Elbow loading was applied to the left forelimb using the procedure described previously (Figure 1) [13]. The lateral and the medial sides of the ulna and humerus were in contact with the loading rod and the stator, respectively. The tip of the loader had a contact surface of 3 mm in diameter, and the loading force at 2 Hz for 5 min was selected in the range of 0.2–4 N. The right forelimb was used as a sham loading control (contralateral control), which was placed under the loader with no dynamic loading.

Strain Measurements

Six mice were used for strain measurements. The medial periosteal surface of the ulna was surgically exposed, and a strain gauge (EA-06-015DJ-120; Vishay Measurements Group, Malvern, PA, USA) was glued on a cortical surface at a location 2.5 mm (metaphysis) or 4.5 mm (diaphysis) apart from the proximal end of the ulna. Voltage signals from the strain gauge were analyzed using a signal-conditioning amplifier and a data acquisition software tool in MatLab (version 6.0, Mathworks, Natick, MA, USA).

Semi-quantitative PCR

Ulna tissue samples (cortical bone and articular cartilage) from 12 mice were harvested 1 hr after elbow loading. Bone and cartilage samples were isolated separatively and homogenized. Total RNA was isolated with an RNeasy mini kit (Qiagen, Hilden, Germany). Using ~50 ng of total RNA, reverse transcription was conducted with high-capacity cDNA reverse transcription kits (Applied Biosystems, Carlsbad, CA, USA). Semi-quantitative PCR was performed to determine the mRNA levels of osteocalcin, MMP-1, MMP-3, MMP-8, MMP-13, MMP-14, TIMP-1, TIMP-2, and CITED2. The PCR primers are listed in Table 1. Thirty-two PCR cycles were employed with a temperature profile at 94°C for denaturation (30 s), 55–65°C for annealing (30 s), and 72°C for extension (45–60 s). GAPDH was used as an internal control.

MMP Activity Assay

Using six mice, MMP activities were determined with fluorophore-labeled substrates selective to MMP-1 and MMP-13 (#444219 and #444235, Calbiochem, Darmstadt,

Germany) as well as gelatinases or collagenases (EnzChekgelatinase/collagenase assay kit E12055, Molecular Probes, Eugene, OR, USA). DQ gelatin and DQ collagen (D-12060, Molecular Probes) were employed to assay activities of gelatinases and collagenases, respectively. A forelimb with an elbow joint was removed, flash-frozen in liquid nitrogen, and stored at −80°C. Elbow articular cartilage was dissected after the frozen limb was thawed on ice. The sample was ground with a mortar and pestle, and a reaction buffer consisting of 50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 5 mM CaCl2, and 0.2 mM sodium azide was added. The proteins isolated from the articular cartilage were incubated with the fluorescent substrates in the reaction buffer at room temperature for 2 hr. Fluorescent intensity, a measure of MMP activity, was determined by a FluoroMax-2 spectrofluorometer (Instruments S.A., Inc., Sunnyvale, CA, USA). An absorption/emission wavelength was set to 382/441 nm (MMP-1), 325/393 nm (MMP-13), and 495/515 nm (collagenases and gelatinases). The MMP activities were normalized using the concentrations of total proteins.

Statistical Analysis

The data were expressed as mean ± SD. A paired *t*-test was employed to evaluate statistical significance between the loaded and control samples. All comparisons were two tailed and statistical significance was assumed for $p < 0.05$.

RESULTS

Strain Induced in the Metaphysis and Diaphysis of the Ulna by Elbow Loading

Prior to examining its loading effects on MMPs in the articular cartilage, we determined load-induced strain in the metaphysis and diaphysis of the ulna. In response to loads at 0.2, 0.5, and 2 N, the ulna metaphysis induced mean strains of 15, 39, and 93 μstrain, respectively (Figure 2A). The strain in the diaphysis was smaller than that in the metaphysis. Its strain was under a detectable level to loads at 0.2 and 0.5 N, while it was ~15 μstrain to 2-N loads (Figure 2B). The strain in the articular cartilage was not directly measured because the strain gauge was unable to be immobilized on the cartilage surface.

Elevation of the Osteocalcin mRNA Level in the Diaphysis of the Ulna

To validate the load-driven anabolic effects on cortical bone in the ulna, we determined the osteocalcin mRNA level in the diaphysis at 24 hr after the loading. Although load-driven strain was undetectably low in the dialysis, the mRNA level of osteocalcin was elevated at the same diaphysis location in response to 0.5 N elbow loading (Figure 2C).

Altered mRNA Levels of MMPs and TIMPs in the Articular Cartilage

Using three levels of loading intensities (0.2, 0.5, and 2 N), we determined the mRNA levels of five MMPs (MMP-1, MMP-3, MMP-8, MMP-13, and MMP-14) and two TIMPs (TIMP-1 and TIMP-2) (Figure 3A). The mRNA levels of MMP-1, MMP-3, MMP-8, and MMP-13 were downregulated by loads of 0.2 and 0.5 N, while the levels of MMP-1 mRNA and MMP-13 mRNA were upregulated by 2-N loads. None of the loads had significant effect on MMP-14 mRNA. The mRNA levels of TIMP-1 and TIMP-2 were unchanged in response to 0.2 and 0.5 N, but they were elevated in response to loads at 2 N.

Induction of CITED2 mRNA in the Articular Cartilage

CITED2 is a transcription regulator that is reported to mediate the suppression of MMP-driven tissue degradation in the articular cartilage [14]. The mRNA level of CITED2 was undetectable in the articular cartilage of the control animal (Figure 3B). However, its mRNA level was elevated in response to 1 N loads. This elevated level of CITED2 expression was reduced by loads at 2 N, and it became undetectably low to higher loads at 4 N.

Load-Driven Alterations in MMP Activities in the Articular Cartilage

We examined four types of MMP activities, using fluorescent substrates selective to gelatinases, collagenases, MMP-1, or MMP-13 (Figure 4). The result revealed that 0.5 N loads downregulated all four types of MMP activities. However, higher loads at 2 N upregulated those MMP activities. Compared to the non-loaded control, for instance, the levels of MMP-1 activity were determined as 0.72 ± 0.13 (mean ± SD; 28% reduction by 0.5 N loads) and 1.73 ± 0.04 (73% elevation by 2 N loads).

DISCUSSION

This study presents that elbow loading not only activates the expression of an anabolic gene in the cortical bone but also regulates the expression of MMPs in the articular cartilage. Although strain induced by elbow loading at 0.2–2 N was smaller than 100 μstrain in the cortical bone, loads at 0.5 N were sufficient to elevate the mRNA level of osteocalcin in the diaphysis. In response to loads at 0.2 and 0.5 N, elbow loading reduced the expression and activities of MMP-1 and MMP-13. On the contrary, it increased their mRNA and degenerative activity levels by higher loads at 2 N. We have previously shown that a natural joint rotation decreases enzyme activities of collagenases [12]. To our knowledge, this study demonstrated for the first time that moderate lateral loads to the synovial joint can suppress degenerative actions of MMPs in the articular cartilage.

The results herein are consistent with intensity-dependent regulation of MMPs in cultured chondrocytes [14]. In vitro MMP data pointed out that fluid shear at 1–10 dyn/cm^2 reduced the expression and activities of MMP-1 and MMP-13, while fluid shear at 20 dyn/cm^2 increased them. This in vivo study also supports the mechanism in which loads below a threshold value decreased proteolytic actions of MMPs. Besides the expression of MMP-1 and MMP-13, we examined load-driven alterations in the mRNA levels of MMP-3, MMP-8, MMP-14, TIMP-1, and TIMP-2. MMP-3 cleaves the non-helical telopeptide regions of type II collagen [15], and it activates other MMPs such as MMP-1 [16]. MMP-8 is a neutrophil collagenase that degrades type I, II, and III collagens [17], and MMP-14 is a membrane-type MMP that is expressed at the cell surface [18]. TIMP-1 and TIMP-2 are natural inhibitors of MMPs [19]. Collectively, the data in this study indicate that joint loading significantly reduced the expression profile of multiple MMPs, that is, MMP-1, MMP-3, MMP-8, and MMP-13, and overall activities of collagenases or gelatinases in articular cartilage, while higher loads increased the expression and activity of MMP-1 and MMP-13. In response to mechanical stimulation, the concomitant elevation of the mRNA levels of TIMP-1 and TIMP-2 as well as MMP-1 and MMP-13 were also reported in synovial cells [20]. It is

possible that these genes with opposing functions are at least in part co-regulated in joint tissues but the mechanism of their transcriptional activation needs to be further investigated.

In load-driven regulation of MMPs, several signaling pathways are considered to be involved in its mechanotransduction and gene expression. Moderate loading can block IL-1β-induced transcriptional activity of NFκB by interfering with multiple steps in the NFκB signaling cascade [21]. High-amplitude loading activates NFκB, which regulates the expression of pro-inflammatory cytokines and mediators such as NOS-2, COX-2, MMPs, TNF-α, and IL-1β [22]. Mechanical overloading also stimulates the expression of VEGF, which appears to be involved in the induction of MMP-1, MMP-3, and MMP-13 expressions [23]. In this study, we focused on the potential linkage to CITED2, a member of the Cited family of transcriptional regulators. An overexpression of CITED2 in cultured chondrocytes repressed the mRNA and activity levels of mRNA of MMP-1 and MMP-13 [14]. Furthermore, gentle joint rotation is reported to induce CITED2 expression and suppress degenerative actions of MMPs [24]. Although direct comparison between CITED2 and MMP mRNA levels is only possible at 2 N in this study, these results together with the in vivo study herein indicate that CITED2 is potentially involved in load-driven downregulation of MMP-1 and MMP-13. However, the results in this study do not exclude a possibility that other signaling pathways are also involved.

This study has several limitations. First, the strain measurement using strain gauges did not enable to detect elbow-loading-induced strain in the articular cartilage. Other methods such as digital image correlation [25] and three-dimensional electrospeckle interferometry [26] together with finite element numerical simulations might be useful to determine in vivo strain. Second, besides articular cartilage the synovial joint includes tissues such as meniscus and synovium [27]. It is important to evaluate MMP expression and activities in other tissues and their dependence on loading intensities. A loading force of 2 Hz for 5 min was chosen since its effects on bone remodeling have been reported [4,5]. However, other loading frequencies may have different effects on the articular cartilage. In response to the same joint loading procedure, it is possible that individual tissues in the joint receive different loading intensities and respond differently. Third, this study mainly focused on the responses 1 hr after the loading. It is important to understand temporal responses such as responses 1 day and 1 week after the loading. MMPs degrade various ECM molecules including type II collagen and proteoglycans, which constitute a majority of the ECM of the articular cartilage. Other degenerative enzymes such as ADAMTS are important and their responses to mechanical loading need to be evaluated [28,29].

In summary, a tight interrelationship exists between bone and cartilage. Loading at a moderate intensity appears to be necessary for normal bone remodeling as well as cartilage maintenance. While the mechanotransduction in these two tissues is distinct, a better understanding of the complex interplay between these two tissues may help us to develop a therapeutic loading strategy that prevents both bone loss and cartilage degeneration. For a case of osteoarthritis in which misalignment of the articular cartilage induces an irregular joint motion, joint loading may provide moderate mechanical stimulation and reduce activities of degenerative enzymes. Knee loading is another form of joint loading, and the examination of the effects of knee loading on the articular cartilage of the tibia and femur

might be a next step to evaluate joint loading as a potential regimen for treatment of osteoarthritis.

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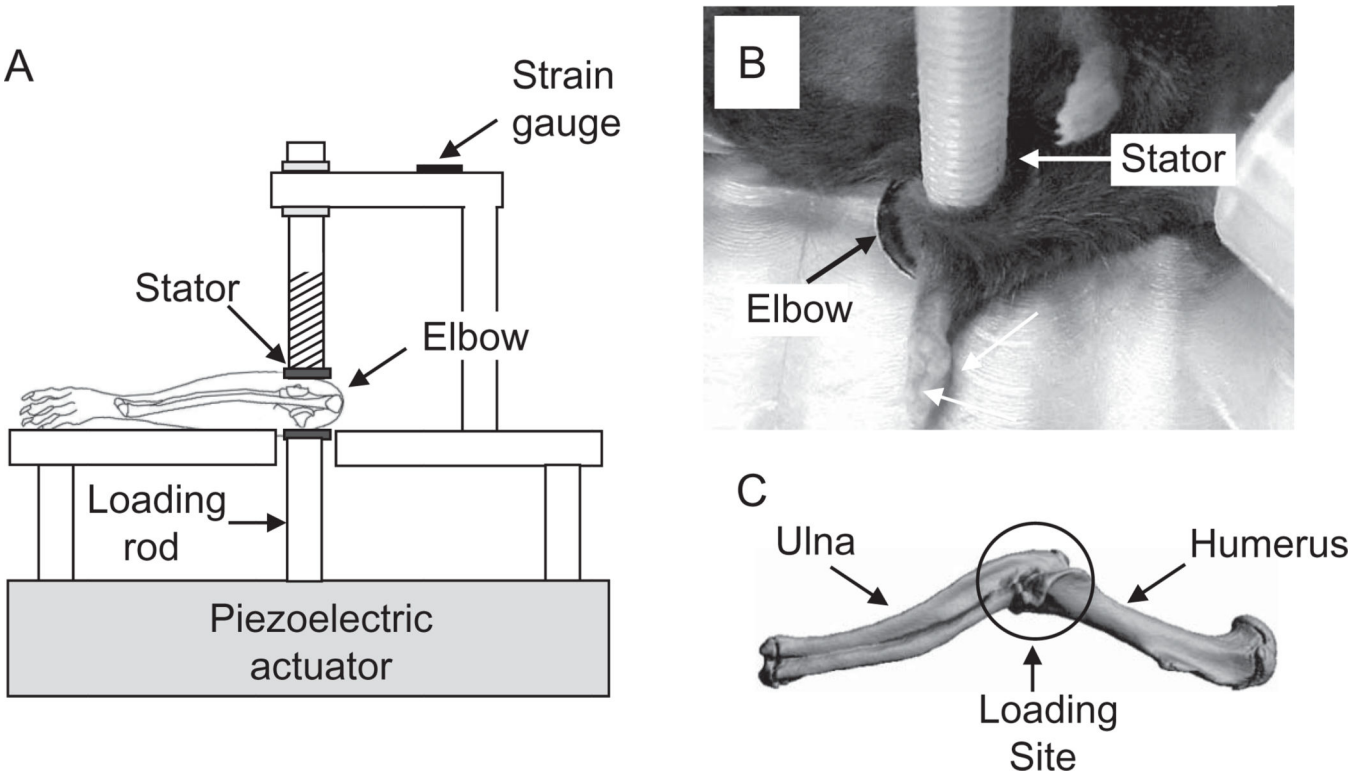

Figure 1.
Experimental setup. (A) Schematic illustration of elbow loading. (B) Mouse mounted for elbow loading. (C) Loading site on the elbow.

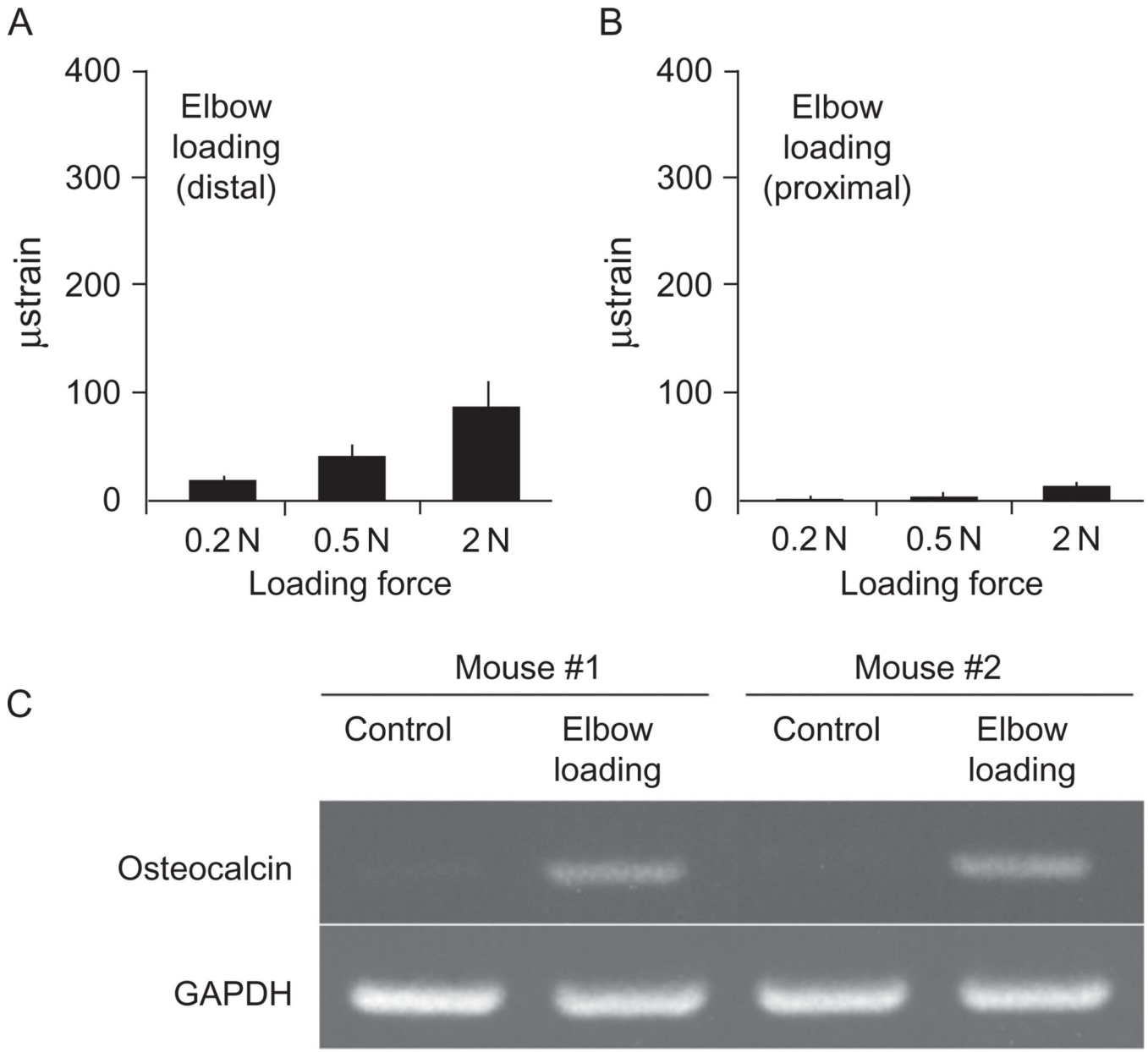

Figure 2.

Effects of elbow loading on cortical bone of the ulna. (A) Strain at 2.5 mm (metaphysis) from the proximal end of the ulna in response to loads at 0.2, 0.5, and 2 N. (B) Strain at 4.5 mm (diaphysis) from the proximal end of the ulna in response to loads at 0.2, 0.5, and 2 N. (C) Osteocalcin mRNA level with and without elbow loading with 0.5 N force.

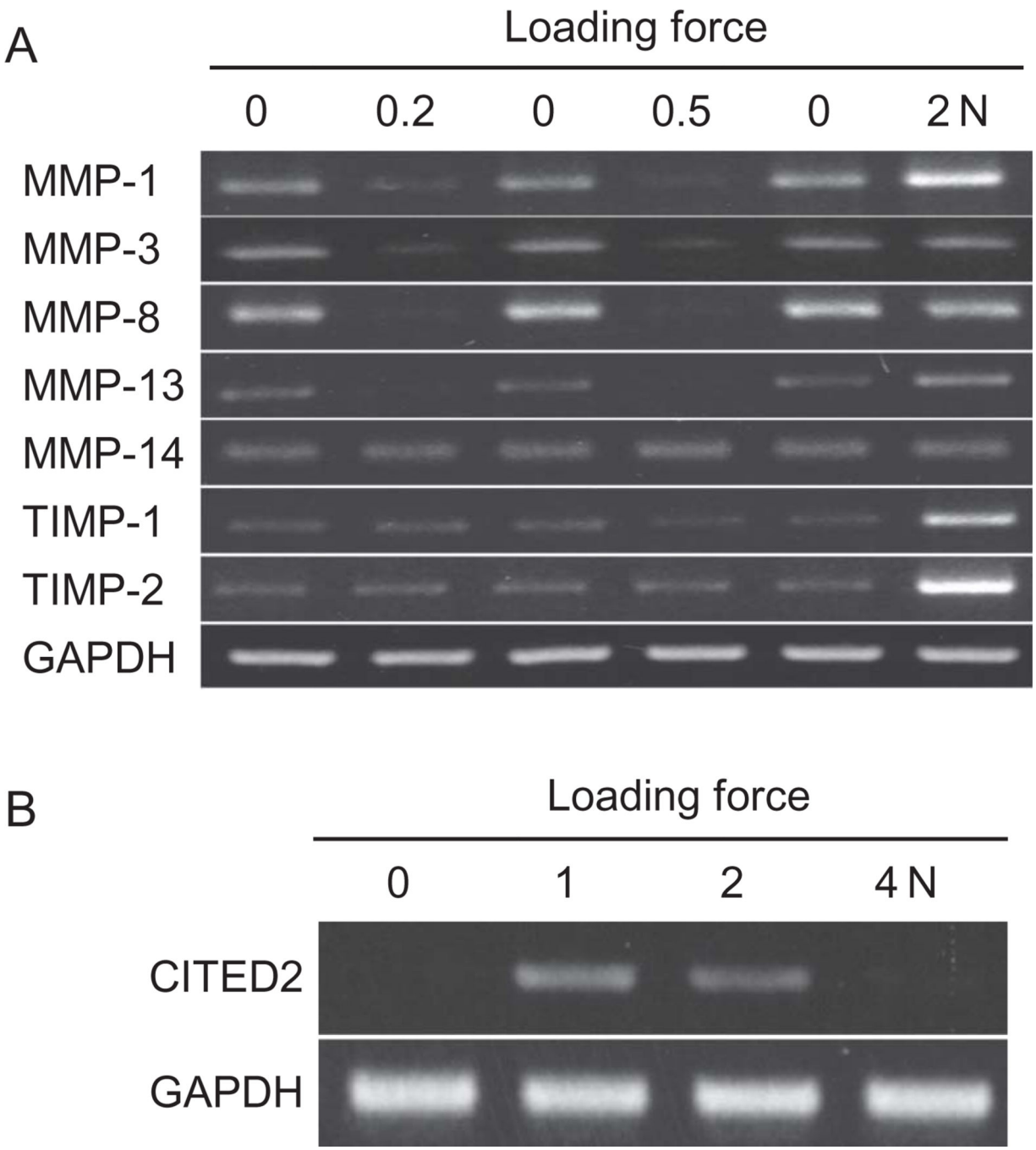

Figure 3.
Alterations in mRNA levels in response to elbow loading. (A) Altered mRNA levels of MMP-1, MMP-3, MMP-8, MMP-13, MMP-14, TIMP-1, and TIMP-2. (B) Altered mRNA level of CITED2.

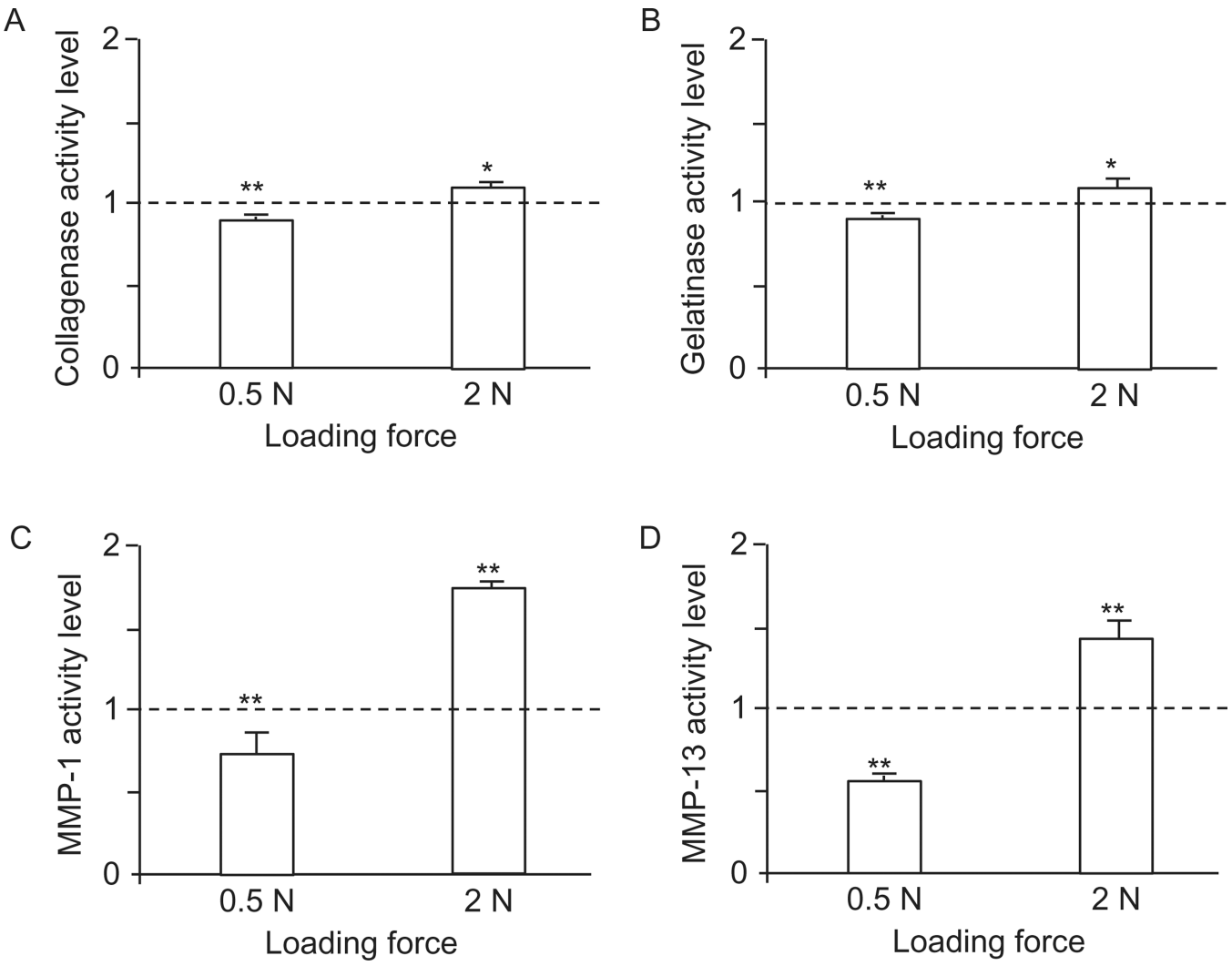

Figure 4.

MMP activities in response to elbow loading. The single and double asterisks indicate $p < 0.05$ and $p < 0.01$, respectively. (A) Gelatinase activity, (B) collagenase activity, (C) MMP-1 activity, and (D) MMP-13 activity.

Table 1

PCR primers used in this study

Gene	Forward primer	Backward primer
MMP-1	5′-ATACACAGTCTATGGATCCA-3′	5′-GGTTCTTCAGTTAATAACTTC-3′
MMP-3	5′-ACCGGATTTGCCAAGACAGAG-3′	5′-AGCCTTGGCTGAGTGGTAGA-3′
MMP-8	5′-TGCCACGATGGTTGCAGAGAAGC-3′	5′-GCCCTTGACAGCTGTGGCGT-3′
MMP-13	5′-CTGGTCTTCTGGCACACGCT-3′	5′-GCAGCGCTCAGTCTCTTCAC-3′
MMP-14	5′-ATGTCTCCCGCCCCTCGACC-3′	5′-GAGGGCGCCTCATGGCCATC-3′
TIMP-1	5′-ACACCAGAGCAGATACCATGATGGC-3′	5′-TATCTGCGGCATTTCCCACAGC-3′
TIMP-2	5′-TGCTGCTAGCCACGCTGCTG-3′	5′-CGAGACCCCGCACACTGCTG-3′
CITED2	5′-AGCACGCCTTCAACGCCCTC-3′	5′-GGCCTGCAGTGGGGTGCAAA-3′
Osteocalcin	5′-GTCCTCCTGGTTCATTTCTTTG-3′	5′-CAGGCTGGCTTTGGAACTTG-3′
GAPDH	5′-GCCACCCAGAAGACTGTGGAT-3′	5′-TGGTCCAGCCTTTCTTACTCC-3′