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# Effects of surgical holes in mouse tibiae on bone formation induced by knee loading

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**Abstract**

Loads applied directly to the knee (knee loading) have recently been demonstrated to induce anabolic responses in femoral and tibial cortical bone. In order to examine the potential role of intramedullary pressure in generating those knee loading responses, we investigated the effects of drilling surgical holes that penetrated into the tibial medullary cavity and thereby modulated pressure alteration. Thirty-nine C57/BL/6 female mice in total were used with and without surgical holes, and the surgical holes were monitored with micro CT and histology. The left knee was loaded for 3 days, and the contralateral limb was treated as a sham-loaded control. Mice were sacrificed for bone histomorphometry 2 weeks after the last loading. Although the surgical hole induced bone formation in both loaded and non-loaded tibiae, due to regional and systemic acceleratory phenomenon the anabolic effect of knee loading was substantially diminished. Without the holes, knee loading significantly elevated cross-sectional cortical area, cortical thickness, mineralizing surface, mineral apposition rate, and bone formation rate on the periosteal surface. For example, the rate of bone formation was elevated 2.1 fold ($p<0.001$; middle diaphysis — 50% site from the knee along the length of tibiae) and 2.7 fold ($p<0.01$; distal diaphysis — 75% site). With the surgical holes, however, knee loading did not provide significant enhancement either at the 50% or 75% site in any of the histomorphometric measurements ($p>0.05$). The results support the idea that alteration of intramedullary pressure is necessary for knee loading to induce bone formation in the diaphysis.


*Keywords:* Loading; Knee; Bone formation; Tibia; Surgical hole; Intramedullary pressure

## Introduction

Mechanical loads can provoke remodeling of bone structure and prevention of bone loss [1–3]. Joint loading is one loading modality recently employed as a useful method for inducing bone formation in laboratory animals. Through lateral loads applied to the epiphysis, it has been shown to elevate anabolic responses in long bones including ulnae [4], tibiae [5], and femora [6]. Compared to ulna axial loading, for instance, joint loading (elbow loading) requires a smaller force (1/4 or less of the force required with ulna axial loading) and induces minute strain (~30 μstrain) at the site of bone formation [7]. Knee loading is one form of joint loading specific to a hindlimb, in which sideward loads are directed to a distal femoral epiphysis together with a proximal tibial epiphysis [5,6]. Since the loading site in the epiphysis and the region of bone formation in the diaphysis are structurally separated, biophysical and/or biochemical mediators likely connect these two locations [8].

Using the mouse hindlimb as a model system, we recently demonstrated that knee loading induces alteration of intramedullary pressure in the femoral bone cavity and that this alteration is synchronous to the loading frequency in Hz [9]. In separate loading studies, augmentation of intramedullary pressure through an external pressure source has also been shown to increase bone formation using *in vivo* turkey ulnae [10,11]. Similarly, a pressure gradient, elevated by venous ligation, was shown to increase interstitial fluid flow and this flow-mediated bone adaptation was considered to be independent of mechanical strain [12–14]. Alteration of intramedullary pressure is therefore likely to be a potential mediator for knee-

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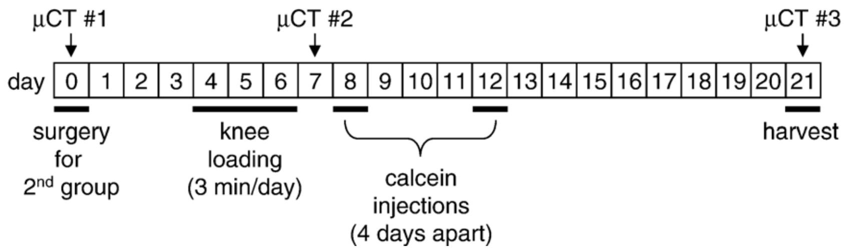


Fig. 1. Time line of the study. μCT = micro computer tomography.

loading-driven bone formation [8]. Herein we examined the role of intramedullary pressure in knee loading using a surgical approach—drilling holes as a means of pressure interference.

Focusing on the middle and distal diaphysis in the tibia, the current study aimed to inquire about the role of intramedullary pressure in load-driven bone formation by drilling a penetrating

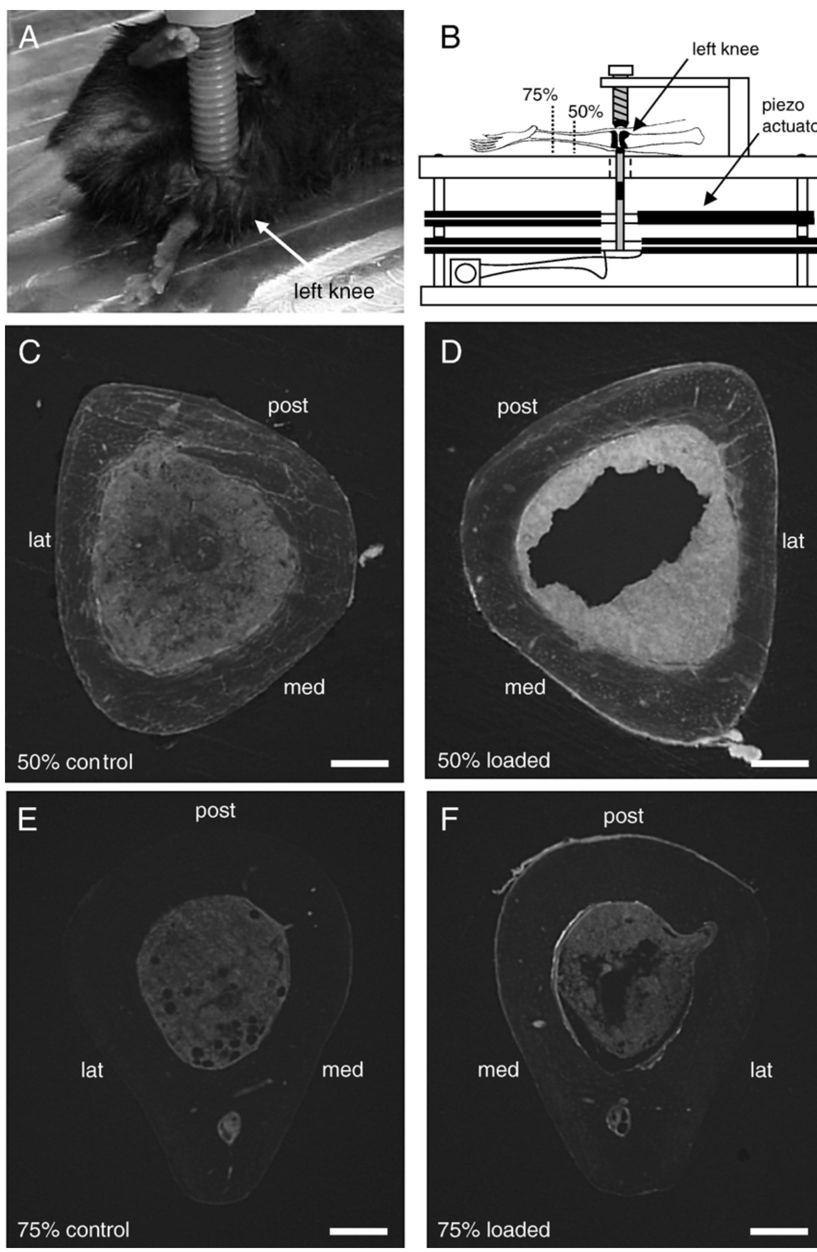


Fig. 2. Knee loading without a surgical hole. (A) Left knee without a surgical hole on a loading table for knee loading. (B) Schematic diagram of the piezoelectric mechanical loader used in this study. Two locations (50% and 70% sites from a tibial proximal end along the length of a tibia) were used for bone histomorphometry. (C) Cross-section of the control tibia at the 50% site. Scale bar = 200 μm in C to F. (D) Cross-section of the loaded tibia at the 50% site. (E) Cross-section of the control tibia at 75% site. (F) Cross-section of the loaded tibia at 75% site. Note that “med” = medial surface; “lat” = lateral surface; and “post” = posterior surface.