Bone 57 (2013) 355–360

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

Chronic skeletal unloading of the rat femur: Mechanisms and functional consequences of vascular remodeling

John N. Stabley [a], Rhonda D. Prisby [b], Bradley J. Behnke [a], Michael D. Delp [a,*]

[a] Department of Applied Physiology and Kinesiology, and the Center for Exercise Science, University of Florida, Gainesville, FL 32611, USA
[b] Department of Kinesiology and Applied Physiology, University of Delaware, Newark, DE 19716, USA

ARTICLE INFO

Article history:
Received 2 July 2013
Revised 14 August 2013
Accepted 11 September 2013
Available online 19 September 2013

Edited by: David Burr

Keywords:
Bone blood flow
Hindlimb unloading
Vascular resistance

ABSTRACT

Chronic skeletal unloading diminishes hindlimb bone blood flow. The purpose of the present investigation was to determine 1) whether 7 and 14 days of skeletal unloading alter femoral bone and marrow blood flow and vascular resistance during reloading, and 2) whether putative changes in bone perfusion are associated with a gross structural remodeling of the principal nutrient artery (PNA) of the femur. Six-month old male Sprague–Dawley rats were assigned to 7-d or 14-d hindlimb unloading (HU) or weight-bearing control groups. Bone perfusion was measured following 10 min of standing (reloading) following the unloading treatment. Histomorphometry was used to determine PNA media wall thickness and maximal diameter. Bone blood flow, arterial pressure and PNA structural characteristics were used to calculate arterial shear stress and circumferential wall stress. During reloading, femoral perfusion was lower in the distal metaphyseal region of 7-d HU rats, and in the proximal and distal metaphyses, diaphysis and diaphyseal marrow of 14-d HU animals relative to that in control rats. Vascular resistance was also higher in all regions of the femur in 14-d HU rats during reloading relative to control animals. Intraluminal diameter of PNAs from 14-d HU rats (138 ± 5 μm) was smaller than that of control PNAs (162 ± 6 μm), and medial wall thickness was thinner in PNAs from 14-d HU (14.3 ± 0.6 μm) versus that of control (18.0 ± 0.8 μm) rats. Decreases in both shear stress and circumferential stress occurred in the PNA with HU that later returned to control levels with the reductions in PNA maximal diameter and wall thickness, respectively. The results demonstrate that chronic skeletal unloading attenuates the ability to increase blood flow and nutrient delivery to bone and marrow with immediate acute reloading due, in part, to a remodeling of the bone resistance vasculature.

Introduction

The chronic unloading of the skeleton, such as occurs with prolonged bedrest and microgravity, induces a number of alterations to bone, including reductions in bone mass, density, mineralization, trabecular thickness and osteoblastic activity [1–4]. Despite the critical role the microcirculation plays in the maintenance of bone interstitial fluid pressure and flow [5–8], osteoblast and osteoclast progenitor cell transport [9,10], and the putative coupling of bone perfusion and vascular signaling to bone remodeling [6,9,11–15], few studies in the literature have examined the effects of chronic unloading on the skeletal vasculature. Fei et al. [17] have reported small vessel rarefaction in the tibia of hindlimb unloaded (HU) rats, but the effects of unloading on the bone resistance vasculature, the site of control of bone and marrow perfusion, are unknown.

In human models of skeletal unloading, disuse osteopenia occurs at sites where hydrostatic pressure is reduced [7,18,19]. Likewise, in patients with unilateral lower limb arterial disease, reductions in blood flow to the affected limb are associated with decrements in femoral bone mineral density [20]. In HU rats, bone loss also occurs in skeletal sites where arterial pressure and perfusion are diminished [1,6]. Although reductions in both arterial pressure and blood flow have been previously shown to induce vascular remodeling of large conduit arteries [21–24] and resistance arteries in skeletal muscle [25], no studies have examined the impact of altered microvascular hemodynamics on resistance artery structure in bone. Therefore, the purpose of the present investigation was to determine, 1) whether vascular remodeling occurs in the principal nutrient artery (PNA) of the rat femur during skeletal unloading, 2) whether alterations in PNA circumferential wall stress or intraluminal shear stress are regulated variables when putative changes in vascular structure occur, and 3) whether possible changes in vascular structure are associated with alterations in bone and marrow perfusion with reloading. We hypothesized that the unloading-induced reductions in hindlimb arterial pressure would diminish PNA circumferential wall stress while reductions in blood flow to the femur would reduce PNA intraluminal shear stress. Further, reductions in these mechanical stresses would be normalized through decreases in PNA medial wall thickness and intraluminal diameter, respectively. Additionally, we hypothesized that the shear stress-induced reductions in PNA intraluminal diameter would be associated with a diminished bone hyperemia with skeletal reloading.

* Corresponding author at: Department of Applied Physiology and Kinesiology, University of Florida, Gainesville, FL 32611, USA. Fax: +1 352 392 5262.
E-mail address: mdelp@ufl.edu (M.D. Delp).

8756-3282/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bone.2013.09.003

Methods

Animals

All procedures performed in this study were approved by the University of Florida and the Texas A&M University Institutional Animal Care and Use Committees and conformed to the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* (Eighth edition, 2011).

Six-month-old male Sprague–Dawley rats ($n = 49$) were obtained from Harlan (Houston, TX) and individually housed in a temperature-controlled (23 ± 2 °C) room with a 12:12 h light–dark cycle. Water and rat chow were provided *ad libitum*. Animals were randomly assigned to either a normal weight-bearing control (Con) group or 7-d and 14-d hindlimb unloaded (HU) groups. The animals were further subdivided into groups for blood flow (Con, $n = 13$; 7-d HU, $n = 11$; 14-d HU, $n = 11$) and arterial morphology (Con, $n = 7$; 14-d HU, $n = 7$) determination. The hindlimbs of the HU groups were elevated to an approximate spinal angle of ~40° via orthopedic traction tape placed around the proximal two-thirds of the tail in a modification of techniques previously described [6,25,26]. The height of the hindlimb elevation was adjusted to prevent the hindlimbs from touching supportive surfaces while the forelimbs maintained contact with the cage floor. This configuration allowed free range of movement around the cage while unloading the hindlimb skeleton. HU animals were kept in this position for a period of 7 or 14 d. Control animals were individually maintained in their normal cage environment.

Bone blood flow determination

Surgical procedures

Control and 7-d and 14-d HU rats were anesthetized with pentobarbital sodium (30 mg/kg ip). HU rats were anesthetized while remaining in the unloaded position to avoid any weight-bearing activity. A catheter (Dow Corning, Silastic; ID 0.6 mm, OD 1.0 mm) filled with heparinized (200 U/ml) saline was advanced into the ascending aorta via the right carotid artery as previously described [6,26]. This catheter was subsequently used for the infusion of radiolabeled microspheres to measure blood flow. A second polyurethane catheter (Braintree Scientific, Micro-renathane; ID 0.36 mm, OD 0.84 mm), used for the withdrawal of a reference blood sample and measurement of hindlimb arterial pressure, was implanted in the caudal artery of the tail and filled with heparinized saline as previously described [6,26,27]. Both catheters were externalized and secured on the dorsal cervical region.

Experimental protocol

Following 24 h of recovery from the surgical procedure, Con and HU animals were instrumented for blood flow determination. Blood flow in the Con group was first measured while the animals were hindlimb unloaded by elevating their hindlimbs to an approximate spinal angle of ~40° for 10 min. The animals were then returned to a normal standing position for 10 min and a second blood flow measure was made using different radiolabeled microspheres. In the HU groups, blood flow was first measured while the animals were in the unloaded position. The rats' hindlimbs were then lowered to a weight-bearing standing position and blood flow was again measured following 10 min of standing. After the microsphere infusions, euthanasia solution (0.4 ml/kg; Euthanasia-5 Solution, Henry Schein Inc.) was infused through the carotid catheter. Femora and soleus muscles were removed from the carcass. The soleus muscle was weighed to determine the efficacy of the unloading treatment. Femora from both hindlimbs were sectioned into three regions, the proximal and distal metaphyses and diaphysis; the femoral marrow was removed from the diaphysis and counted as a fourth region as previously described [6,12,15]. Corresponding femoral sections from the left and right hindlimbs were combined for each animal to ensure sufficient microspheres in the samples. Bone samples were weighed and placed in counting vials for flow determination. Mass of the femoral marrow was determined by weighing the shaft before and after the marrow was removed.

Blood flow and vascular resistance

Radiolabeled (^{85}Sr, ^{113}Sn, and ^{46}Sc) microspheres (Perkin Elmer) with a 15 μm diameter were used for blood flow measurements as previously described [6,12,15,26]. The 15 μm diameter of the microspheres results in the spheres being trapped in small arterial vessels upstream of capillaries, and therefore reflects flow through the arteries. Radioactivity of the samples was measured with a gamma counter (Packard AutoGamma 5780) and flows were computed (PC-GERDA V2.9 Software) from counts per minute and tissue wet weights. Vascular resistance was calculated by dividing arterial pressure by tissue blood flow.

Central hemodynamics

Electronically averaged mean arterial pressure and heart rate (pulse pressure rate) were recorded from the caudal catheter immediately before and after each microsphere infusion and averaged.

Arterial morphology determination

Microvessel preparation

At the end of the experimental period, Con and 14-d HU rats were injected with euthanasia solution (0.5 ml/kg ip; Euthanasia-5 Solution, Henry Schein Inc.). The hindlimbs were removed from the carcass and placed in a 4 °C filtered physiological saline buffer solution (PSS). Using a stereomicroscope, the femoral PNA was identified. In the area where the PNA entered the femoral diaphysis, muscle fibers surrounding the bone and PNA were carefully dissected away (Fig. 1). The femoral diaphysis was then cut adjacent to the nutrient foramen and canal using a small

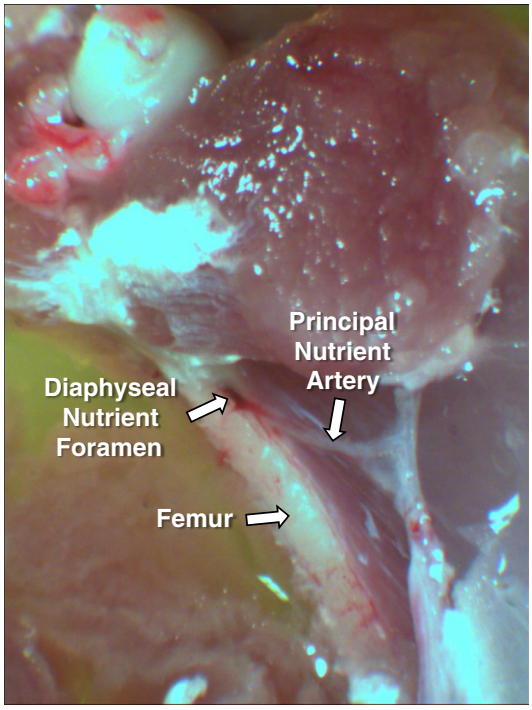

Fig. 1. Photomicrograph of the rat principal nutrient artery and the diaphyseal nutrient foramen of the right femur.