Journal of Biomechanics 45 (2012) 2046–2049

Contents lists available at SciVerse ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Short communication

# Trabecular bone response to mechanical loading in ovariectomized Sprague-Dawley rats depends on baseline bone quantity

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ARTICLE INFO

*Article history:*
Accepted 13 May 2012

*Keywords:*
High baseline BV/TV
Low baseline BV/TV
Mechanical loading
In vivo μCT
Osteoporosis

ABSTRACT

Mechanical loading is one of the determining factors for bone modulation, and is therefore frequently used to treat or prevent bone loss; however, there appears to be no data on the effects of baseline bone quantity on this response. This study aimed to verify whether baseline bone quantity affects osteoporotic trabecular bone adaptive response to mechanical stimulation.

Twenty-four female Sprague-Dawley (SD) rats were ovariectomized (OVX). After 3 weeks of OVX, rats were divided into a high bone quantity and a low bone quantity group, and rats in each group were then subdivided into 4 groups that were exposed to different loading strategies. In the loading groups, tibiae were stimulated through axial loading at 2000 με of strain, for 1500 cycles each of 75 s, 150 s, or 250 s. The sham treatment groups received no loading.

Changes in BV/TV for trabecular bone in the tibia were measured at the baseline (before loading), and at 3 weeks and 6 weeks after loading. BV/TVs in loading groups of the low baseline bone quantity group were significantly increased at 6 weeks, compared with those in the no-loading groups ($p<0.05$), while those in the high quantity groups were not increased ($p>0.05$). A significant negative correlation was observed between baseline BV/TV and its relative variations at 3 weeks or 6 weeks ($p<0.05$).

These results indicate that adaptive responses of osteoporotic trabecular bone to mechanical loading depend on baseline bone quantity.



## 1. Introduction

Mechanical loading is an important factor for regulating bone adaptation (Frost, 2004; Ko et al., 2011; Qin et al., 1998; Turner, 1998). Based on these factors, previous studies have attempted to verify the feasibility of mechanical loading for the treatment osteoporosis. However, the reported effects of mechanical loading on osteoporosis have been varied widely (Lynch et al., 2010; Lynch et al., 2011; Prisby et al., 2008; Totosy de Zepetnek et al., 2009; Turner et al., 2011; van der Jagt et al., 2012). The reason for diverse effects of mechanical loading is unclear, but differences in baseline bone quantity may be responsible.

Previous studies suggested that bone adaptive responses to mechanical loading depend on its parameters (Jarvinen et al., 2003; Judex et al., 2007; Turner et al., 1995) as well as age and genetics (De Souza et al., 2005; Robling and Turner, 2002; Robling et al., 2007) of subjects applied by loading. However, to our knowledge, few studies have reported the effects of baseline bone quantity on the response of bone to mechanical loading in rats of an identical animal strain.

This study aimed to verify whether baseline trabecular bone quantity affected the response of trabecular bone to mechanical loading on osteoporotic bone.

## 2. Methods

All procedures had been approved by the Yonsei University Animal Care Committee.

Twenty-four female Sprague-Dawley (SD) rats (12-week-old, $242.1\pm5.5$ g) were ovariectomized (OVX). After 3 weeks, trabecular bone loss in the tibia of rats was verified through changes in bone volume fraction (BV/TV [%], 47.5% decrease).

At this stage, rats were divided into 2 groups: high BV/TV (H, $28.4\pm4.3$%) and low BV/TV (L, $7.3\pm3.5$%) groups; rats with $>50$% BV/TV of all rats were categorized under the H group and the others, under the L group. Rats in each group (H and L) were further subdivided into 4 subgroups (Table 1): 3 loading and 1 sham (S) treatment group. In the loading groups, the right tibia of each rat was stimulated through axial loading at 2000 με strain (the compressive displacement of the actuator, supplementary information 1 and Fig. 1), as described previously

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0021-9290/$ - see front matter 
http://dx.doi.org/10.1016/j.jbiomech.2012.05.013

**Table 1**
Descriptive data of BV/TV (%). Data are given as mean ± standard deviation.

| | L-250 s | H-250 s | L-150 s | H-150 s | L-75 s | H-75 s | L–S | H–S |
|---|---|---|---|---|---|---|---|---|
| **0 weeks** | 5.55 ± 2.60 | 30.49 ± 3.16$^{*}$ | 6.06 ± 2.44 | 31.43 ± 3.73$^{*}$ | 5.99 ± 3.17 | 25.68 ± 2.34$^{*}$ | 11.57 ± 2.81 | 26.02 ± 5.88$^{*}$ |
| **3 weeks** | 4.07 ± 3.24 | 13.19 ± 2.92$^{a,*}$ | 5.96 ± 1.46 | 11.62 ± 2.71$^{a,*}$ | 4.74 ± 2.87 | 10.03 ± 2.25$^{a}$ | 7.51 ± 2.07 | 14.65 ± 4.64$^{a,*}$ |
| **6 weeks** | 8.24 ± 4.39$^{b}$ | 13.63 ± 2.79 | 11.12 ± 3.06$^{b}$ | 13.13 ± 2.71 | 9.13 ± 5.64 | 12.59 ± 3.33 | 8.98 ± 2.38 | 9.23 ± 2.41$^{b}$ |

L: baseline low bone quantity, H: baseline high bone quantity, 75 s: loading for 75 s, 150 s: loading for 150 s, 250 s: loading for 250 s, S: Sham,

$^{*}$ vs. L ($p<0.05$),
$^{a}$ vs. 0 week ($p<0.05$),
$^{b}$ vs. 3 weeks ($p<0.05$).

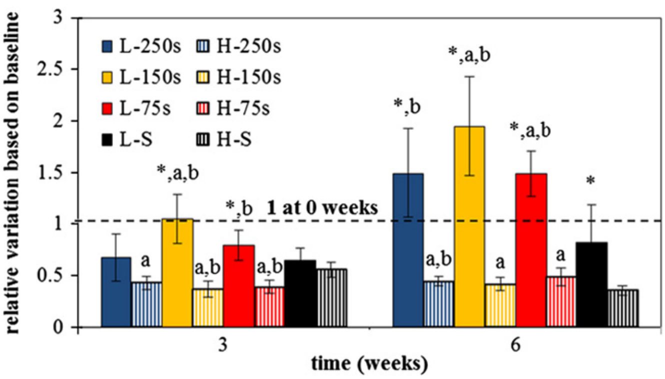


**Fig. 1.** Relative variations in BV/TV based on values at 0 weeks (baseline). Data are given as mean ± standard deviation. L: baseline low bone quantity, H: baseline high bone quantity, 75 s: loading for 75 s, 150 s: loading for 150 s, 250 s: loading for 250 s, S: Sham treatment, $^{*}$ vs. H ($p<0.05$), a: vs. L–S ($p<0.05$), b: vs. H–S ($p<0.05$).

(Mosley et al., 1997; Torcasio et al., 2012), for 1500 cycles of 75 s, 150 s, or 250 s duration each (75 s, 150 s, and 250 s groups). Additionally, rats in both the S groups (the L–S and H–S groups) underwent sham treatment, in which no load was applied.

Tibiae were scanned using in vivo μCT (Skyscan 1076; SKYSCAN N.V., Belgium) before and after 3 and 6 weeks of loading. From these scans, BV/TVs were measured using CT-AN (SKYSCAN N.V.).

A two-way ANOVA was performed to analyze the effect of mechanical loading or the time-mechanical loading interaction. To compare the differences in BV/TV, relative variations (RVs) were calculated as BV/TVs at 3 and 6 weeks and divided by the value at 0 weeks (the RV was set at 1 at 0 weeks). The RV of BV/TV at each time point was compared among each group using the Kruskal–Wallis test. A Pearson correlation test was performed using BV/TV at 0 weeks as an independent variable and the corresponding RV at 3 weeks or 6 weeks as the dependent variable. All statistical analyses were performed using SPSS 17.0 (SPSS Inc., USA) ($p<0.05$).

## 3. Results

The changes in BV/TV over time are shown in Table 1. At 3 weeks, BV/TVs in the H group had significantly decreased ($p<0.05$), but no significant change was observed in the L group ($p>0.05$); however, BV/TVs in the H group were still significantly greater than those in the L group ($p<0.05$). In contrast, at 6 weeks, most BV/TVs in the L groups, except for L-75 s, had significantly increased compared with those at 3 weeks ($p<0.05$), but these changes were not observed in the H groups ($p>0.05$). Moreover, there were no significant differences between the L and H groups at 6 weeks ($p>0.05$).

The differences in RV are shown in Table 2 and Fig. 1. At 3 weeks, the RV of BV/TV in the L-150 s group was significantly higher than that in both S groups ($p<0.05$). The RV of BV/TV in the L-75 s group, on the other hand, was significantly higher than that of the H–S group ($p<0.05$), but not higher than that of the L–S group ($p>0.05$). Furthermore, no significant differences were observed in the RV of BV/TV between the L-250 s group and either of the S groups ($p>0.05$).

However, the RVs of BV/TV in the H-loading groups were significantly smaller than those in the L–S group, except for both the 75 s groups ($p<0.05$). Moreover, in the H-75 s and H-150 s groups, the RVs of BV/TV were significantly smaller than those in the H–S group ($p<0.05$).

There were also significant differences in the RVs of BV/TV between the L and H groups, regardless of loading ($p<0.05$). At 6 weeks, the RVs of BV/TV in L groups were significantly higher than those in the H–S groups, while no significant difference was observed between the L-250 s and L–S groups ($p>0.05$). The RVs of BV/TV were significantly smaller in the H-loading groups than in the L–S groups ($p<0.05$). Furthermore, there were significant differences in the RV of BV/TV between the L and H groups, regardless of loading ($p<0.05$).

Although BV/TVs in both S groups tended to decrease over time, the decrement rate of BV/TV was significantly lower in the L–S group than in the H–S group ($p<0.05$). The differences in BV/TV are shown in Fig. 2. Significant negative correlations were also observed between baseline BV/TV and its RV at 3 weeks and 6 weeks ($R=-0.76$ and $-0.89$, respectively; $p<0.05$), as shown in Fig. 3.

## 4. Discussion

In our study, although progressive bone loss in the L-loading groups occurred at 3 weeks, both the magnitude and rate of bone loss were smaller compared to those of the non-loading groups, except for L-250 s. At 6 weeks, RVs of BV/TV in the L-75 s and L-150 s were greater than those in the non-loading groups ($p<0.05$). However, the RVs of BV/TV in the H-loading groups had not increased compared to those values in the non-loading groups.

Furthermore, changes in BV/TV in the groups exposed to different loading periods (75 s, 150 s, and 250 s) did not differ, regardless of baseline BV/TV. This result was consistent with those of previous studies that showed that the bone response to mechanical loading might also depend on the number of cycles/day (Cullen et al., 2001); a constant 1500 cycles/day in this study. Additionally, it has been reported that the effect of mechanical loading at 2000 με presented at 36 cycles/day did not differ from that at 1800 cycles/day (Rubin and Lanyon, 1984).

Our results suggest that mechanical loading may be effective for the treatment of OVX-induced osteoporosis in cases where baseline BV/TV is low, but not in cases where baseline BV/TV is high. Furthermore, there was a negative correlation between baseline BV/TV and its RVs at 3 weeks or 6 weeks. Together, these data suggest that bone adaptive response to mechanical loading may depend on baseline BV/TV, regardless of an identical strain (SD rats), age (12-week-old at baseline), or gender (all rats were females). Our results may indeed explain the diverse effects of mechanical loading for treating osteoporotic bones; positive (Prisby et al., 2008; Totosy de Zepetnek et al., 2009; Turner et al.,