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Journal of Orthopaedic Research 23 (2005) 188–195

Journal of Orthopaedic Research

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Modulation of vertebral and tibial growth by compression loading: diurnal versus full-time loading

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Received 3 November 2003; accepted 11 June 2004

Abstract

Purpose: This study was designed to determine whether the amount of endochondral growth response to mechanical compression and the underlying growth mechanism differed with night-time or day-time loading, relative to full-time loading.

Methods: Mechanical compression (nominally 0.1 MPa stress) was applied across tibial and tail vertebral growth plates of growing Sprague–Dawley rats. Four groups of animals (five per group) were used: 24/24 h (full-time loading); 12/24 h (day-loading); 12/24 h (night-loading); and 0/24 h (sham instrumented). Contralateral tibiae and adjacent vertebrae served as within-animal controls. The animals were euthanized after eight days. Growth plates were processed for quantitative histology to measure 24-h growth, total and BrdU-positive proliferative zone chondrocyte counts, and hypertrophic chondrocytic enlargement in the growth direction.

Results: Growth as a percentage of within-animal control averaged 82% (full-time); 93% (day-loading); 90% (night-loading); 100% (sham) for vertebrae. For proximal tibiae it averaged 70% (full-time); 84% (day-loading); 86% (night-loading); 89% (sham). Reduced amount of hypertrophic chondrocytic enlargement explained about half of this effect in full-time loaded growth plates, but was not significantly altered in half-time loaded growth plates. The remaining variation in growth was apparently explained by reduced total numbers of proliferative zone chondrocytes. These findings indicate that sustained compression loading suppressed growth more than intermittent loading at both anatomical locations.

Keywords: Growth plate; Biomechanics; Bone; Histology; Chronobiology; Mechanical loading

Introduction

It is thought that progression of deformities such as scoliosis and tibia vara in growing children is caused at least in part by asymmetrical forces on the growth plates, creating differential growth and subsequent deformity [4,14,18]. Conservative management of these deformities by bracing is based on the premise that endochondral growth can be altered beneficially by modifying the mechanical forces acting on the growth plate. Recently, night-time bracing has been proposed for treating scoliosis, as it has been successful in orthodontic bracing [7]. The response of bone growth to loading, and to diurnal variations in loading is not known quantitatively. Equally, the relationship between the timing of the mechanical stimulus relative to the growth response is not known.

Longitudinal growth of long bones and vertebrae occurs in growth plates, where new cells produced in the proliferative zone enlarge and synthesize

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0736-0266/$ - see front matter
doi:10.1016/j.orthres.2004.06.012

extracellular matrix in the hypertrophic zone, and the resulting tissue is then replaced by bone [8,21]. Longitudinal growth can be considered as the product of the rate of new cell production in the proliferative zone, and the amount of cell enlargement in the hypertrophic zone, providing that the thickness of extracellular matrix in the growth direction between cells is ignored. For longitudinal (linear) growth, the relationship

$$\text{growth} = \text{new cells/day} \times \text{final height} \quad (1)$$

represents this presumed relationship between growth and the measured activity of cell production in the proliferative zone and cell enlargement in the hypertrophic zone. The overall height of the growth plate is often considered to be a rough indicator of the rate of longitudinal growth, and it could be considered that this dimension (all other factors being equal) is approximately the product of the total number of active chondrocytes and their size, suggesting that growth plate height might also correlate with variations in growth rate. However, investigations of growth plate kinetics [10,12,21] indicate that more specific measures of chondrocytic activity are required to explain differential growth. Matrix synthesis is also often considered to be an indicator of growth plate activity. Matrix synthesis in the hypertrophic zone is required primarily to create the matrix between cells (perpendicular to the growth direction), since there is generally little matrix observed between hypertrophic chondrocytes in the direction of growth. Similarly, this variable has not been identified as having a key role in regulating growth [10,12].

Hence, we anticipated that for small percentage differences (Δ) produced by mechanical loading, the first order approximation

$$\Delta\ \text{growth} = \Delta\ \text{new cells/day} + \Delta\ \text{Final height} \quad (2)$$

would describe the incremental relationship between in the measurements of loaded relative to within-animal control growth plates.

The aim of this study was to determine whether the amount of endochondral growth response to mechanical compression and the underlying mechanism differed with night-time or day-time loading, relative to full-time loading. The tail vertebral growth plates and in the more rapidly growing proximal tibial growth plates of young rats were studied with nominally 0.1 MPa compressive stress. The null hypothesis was that the growth modulation effect of half-time loading would be half that of full-time loading, irrespective of whether the half-time loading was imposed during the day or night. Also, we investigated the relative contributions of changes in chondrocytic proliferation and of changes in chondrocytic enlargement to the mechanically induced growth modulation.

Methods

Mechanical compression was applied across the growth plates of the seventh caudal (Cd7) vertebra and of the right proximal tibia of 35-day-old Sprague–Dawley rats (Fig. 1). The external loading apparatus used compression springs in an Ilizarov-style construct. Two sterilized stainless steel pins were inserted to transect, respectively, the sixth and eighth caudal vertebrae, the right tibial proximal epiphysis and the right tibial diaphysis. The tail vertebrae pins were 0.5 mm diameter inserted percutaneously and the tibial pins were 0.3 mm diameter, with the epiphyseal pins inserted under direct visualization by surgical exposure of the ventral aspect of the tibia with a midline skin incision. The apparatus was installed under general anesthesia (Ketamine 80 mg/kg and Xylazine 10 mg/kg) with post-operative pain control (Buprenorphine 0.05 mg/kg).

The pins were attached to external plates, and springs were placed on rods linking the plates (Fig. 1). The spring lengths were adjusted to apply forces whose magnitude, divided by the estimated area produced nominally 0.1 MPa compressive stress. The area (A mm^2) was estimated for each rat by using the presumed power-law relationships:

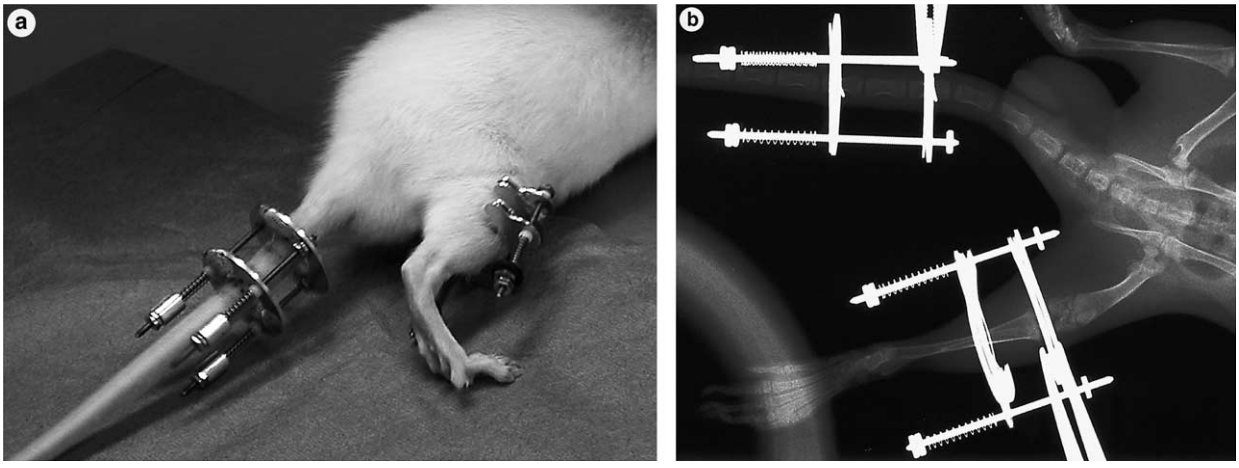

Fig. 1. Photograph (left) and radiograph (right) of an animal with apparatus installed to apply compressive stress to the growth plates of the right proximal tibia and a caudal vertebra. In the radiograph, spacers that are visible in the photograph have been removed to relax the spring forces.

$$A_{\text{tibia}} = 17.3 * (W/125)^{2/3}; \quad \text{and } A_{\text{vertebra}} = 9.9 * (W/125)^{2/3} \tag{3}$$

where W was the bodyweight of the animal in grams and 17.3 and 9.9 were the mean areas measured directly from tibiae and vertebrae, respectively, removed from rats having an average weight of 125 g.

Four groups of animals were studied: 24/24 h (full-time loading); 12/24 h (day-loading); 12/24 h (night-loading); and 0/24 h (sham instrumented with springs left loose), with five animals per group. In half-time loaded animals, spring forces were removed or applied at 'lights-on' and 'lights-off' times (12 h apart) in an artificial light cycle to which animals had previously become acclimated. Animals were euthanized at 'lights-on' on the seventh day after application of the loading apparatus. Calcein (15 mg/kg) was administered systemically 24 h prior to euthanasia, and BrdU (25 mg/kg) 30 min before death. All live animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee.

After euthanasia, the loaded and within-animal control growth plates were excised. For vertebrae both of the loaded (Cd7) growth plates were used, and the within-animal control growth plates were the caudal and cephalad growth plates of Cd5 and Cd9, respectively. For the tibiae, the contralateral (left) proximal growth plate served as control. Two blocks of each growth plate were prepared by cutting each in its sagittal plane, using a razor blade. Each block was fixed [9], dehydrated first in serial alcohols and then in propylene oxide, and embedded in Epon-Araldite using serial dilutions in propylene oxide and vacuum. Sections of each block were cut (1.5 μm thick), mounted, and imaged at 1300×1030 pixel resolution using a Zeiss 'Axioskop' microscope with 10×, 20× or 40× lens. The microscope stage was rotated to align the presumed growth direction with the image frame. Measurements of the images were then made by 'clicking' on image features with a computer 'mouse'. The recorded points were processed by means of custom computer software that took the image magnification (microns per pixel) into account.

Twenty-four hour growth was measured from the fluorescent Calcein label, using a series of points on the labeled bone, and a series of points on the chondro-osseous junction. The mean spacing between spline curves that were fitted numerically to each set of recorded points represented 24-h growth.

In the proliferative zone, each cell nucleus was counted as either BrdU-positive or BrdU-negative, in slides processed to label nuclei containing BrdU (Histostain Sp kit, Zymed Labs, San Francisco, CA). The total number of proliferative chondrocytes with a visible nucleus (N_{prolif}) was the sum of these two counts. The labeling index (LI) was expressed as the number of chondrocytes with BrdU-positive nuclei divided by the total number of cell profiles with visible nuclei [6]. These counts were made in images of a field width equal to 340 μm.

Hypertrophic chondrocytes were identified in stained sections (periodic acid; basic Fuschin; methylene blue; Azure II) by using a semi-automated process employing Zeiss KS-300 software (Carl Zeiss Vision, München, Germany). First, the upper and lower zonal boundaries were identified manually. This region of interest was segmented into dark/light pixels, contiguous zones were identified automatically and identified as chondrocytes if they had a 'form factor' ($4\pi *$ area, divided by squared circumference) greater than 0.3. In a final manual check, regions that represented non-viable or partially sectioned or coalesced cells were eliminated. Chondrocytic enlargement in the growth direction was estimated from the regression relationship of the measured cell height (h) as a function of its vertical position (y) in the hypertrophic zone using the logistic regression curvefit

$$h = a/\left(1 + e^{((m-y)/s)}\right) \tag{4}$$

where a, m, and s are parameters for each analyzed image, evaluated by using the Matlab (Mathworks, Natick MA) least-squares algorithm 'fminsearch.m'. The maximum hypertrophic chondrocytic height ($h_{\max}$) was estimated as the value of h at the at the chondro-osseous junction (Fig. 2). This method was modified from that of Stokes et al. [17] by using a logistic rather than linear regression relationship to represent any non-linear relationship between cell height and position. Growth plate height was recorded as the sum of the proliferative and hypertrophic zonal heights.

Measurements at several spatially separated locations for each growth plate were averaged. Based on initial variance estimates for each measurement, the number of sections averaged was 12 for growth (Calcein label); 16 for proliferative zone measurements (BrdU labeled

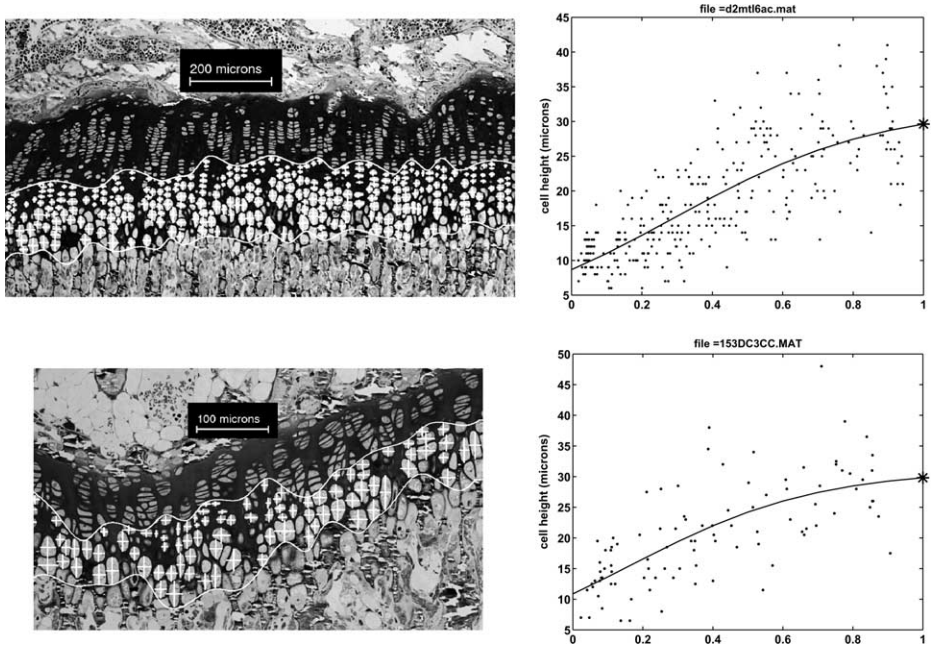

Fig. 2. Left panels: Sections of a rat growth plates (upper, proximal tibial; lower, tail vertebral), with the hypertrophic zone marked as a region of interest by lines at its superior and inferior margins. Cells within this zone have been identified and marked by horizontal and vertical lines. Right panels: graphs of corresponding hypertrophic chondrocytes' heights (vertical axis) versus their position relative to the zonal boundaries (0 = proliferative/hypertrophic boundary; 1 = lower boundary). The fitted line represents the logistic regression (see text). The intercept of this line with the right axis was used as the estimated final chondrocytic height.

nuclei), and five for the final hypertrophic chondrocytic height measurements. The averaged values of loaded and control growth plates for each animal were differenced and expressed as a percentage of the control value.

Growth and final height were measured directly. A relative measure of the new cells per day was obtained from the product of the total number of proliferative chondrocytes and the BrdU labeling index. Hence, it was expected (based on Eq. (2)) that:

$$\Delta \text{ growth} = \Delta\ N_{\text{prolif}} + \Delta\ \text{LI} + \Delta h_{\max} \quad (5)$$

The experimental data were examined by analysis of variance to identify groupwise differences, by 2-tail *t*-tests to identify significant differences between loaded and control growth plates, and by correlation analyses to determine whether there were significant associations between growth and the histological measurements thought to be responsible for growth differences.

Results

Mean growth rates were 38 and 242 microns/day for control vertebral and proximal tibial growth plates, respectively (Table 1). Thus the rate of growth differed by a factor of about 6.4. In these control growth plates, the final chondrocytic height $h_{\max}$ and the BrdU labeling index (LI) were slightly greater for the tibial growth plates, and these two locations differed by a factor of about 2 in number of proliferative cells counted per unit width of growth plate (Table 1).

The measured 24-h growth rate was less in loaded growth plates, the difference between loaded and control sides being significantly different for all loaded tibial growth plates (Table 1). As a percentage of the within-animal control, vertebral growth averaged 82% (full-time group); 93% (day-loading group); 90% (night-loading group); 100% (sham group). For proximal tibiae, the percentage of within-animal control growth averaged 70% (full-time); 84% (day-loading); 86% (night-loading); 89% (sham) (Table 2 and Fig. 3).

There were significant groupwise differences (by analysis of variance) in the growth rates of the tibial growth plates, this being associated (as determined by post hoc comparisons) with significantly lesser growth in the full-time loaded growth plates. In the sham tibial growth plates, reductions were observed in the growth rate and the histological measures. Therefore, in Table 2 the mean percentage differences between loaded and control growth plates are given, along with the differences from the mean values obtained from the sham group. These values indicate that there was a comparable percentage of mechanical growth suppression at both tibial and vertebral locations.

The reduced growth in loaded growth plates corresponded to an observed lesser final chondrocytic height ($h_{\max}$) and reduced counts of proliferative cells (N_{prolif}) in full-time loaded growth plates, although with part-time loading these histological differences were not evident. There were significant differences between loaded and control growth plates in the $h_{\max}$ of both tibiae and vertebrae in the full-time loaded animals, and in the number of proliferative cells counted (N_{prolif}) in the tibial growth plates (Table 1). In groupwise comparisons, $h_{\max}$ differed significantly between groups of tibial growth plates. The proportion of BrdU-positive cells (LI) was observed to be increased relative to internal controls in the loaded growth plates (Table 2, and Fig. 3), but none of these differences was statistically significant. The growth plate height was over two times greater for tibiae, and the operated growth plates had lesser height in all groups except sham vertebrae (Table 1). Groupwise differences were significant for the tibiae. The percentage differences in growth plate height (Table 2) generally did not explain growth rate differences better than the more specific measures of chondrocytic activity $h_{\max}$ and N_{prolif}.

Table 1
Mean (±S.D.) values for loaded and control caudal vertebral and proximal tibial growth plates, in each loading regimen group

	Growth/day (μm)		$h_{\max}$ Final height (μm)		BrdU labeling index LI (%)		N_{prolif} (arbitrary units)		Growth plate height (μm)	
	Loaded	Control	Loaded	Control	Loaded	Control	Loaded	Control	Loaded	Control
Data for vertebrae										
Sham	45 ± 5	46 ± 7	29 ± 2	31 ± 2	7 ± 3	9 ± 3	35 ± 3	35 ± 2	179 ± 14	152 ± 14
Full-time	27 ± 10	33 ± 11	26 ± 2*	23 ± 2*	8 ± 3	8 ± 3	34 ± 4	38 ± 4	130 ± 7	156 ± 10
Day	34 ± 4	37 ± 5	28 ± 3	28 ± 5	8 ± 4	6 ± 2	55 ± 8	62 ± 12	153 ± 14	160 ± 12
Night	32 ± 6	35 ± 7	26 ± 3	27 ± 2	8 ± 5	7 ± 3	62 ± 8	64 ± 15	143 ± 20	152 ± 28
Mean		38 ± 8		28 ± 3		7.4 ± 2.8		49 ± 16		152 ± 16
Data for tibiae										
Sham	224 ± 22	252 ± 22	30 ± 3	32 ± 2	10 ± 2	10 ± 3	81 ± 3	84 ± 6	405 ± 61	448 ± 46
Full-time	162 ± 41**	231 ± 35**	28 ± 2*	30 ± 3*	11 ± 2	10 ± 3	67 ± 14*	79 ± 7*	301 ± 58	393 ± 51
Day	200 ± 13**	239 ± 9**	31 ± 2	30 ± 1	12 ± 3	10 ± 3	107 ± 27	110 ± 29	329 ± 56	355 ± 47
Night	208 ± 9**	242 ± 13**	29 ± 2	29 ± 1	6 ± 3	6 ± 3	115 ± 23	120 ± 15	317 ± 24	348 ± 25
Mean		242 ± 20		30 ± 2		9.4 ± 3.3		97 ± 23		382 ± 54

* Significant difference between the loaded and control paired growth plates ($p < 0.05$).
** Significant difference between the loaded and control paired growth plates ($p < 0.01$).

Table 2
Mean (S.D. in parentheses) of growth and measures of chondrocytes in the proliferative and hypertrophic zones, expressed as a percentage of within-animal control values

	Vertebrae					Tibiae				
	Growth	h_{max}	LI	N_{prolif}	Growth plate height	Growth	h_{max}	LI	N_{prolif}	Growth plate height
Sham	100 (6.2) [0]	96 (5.7) [0]	82 (12.9) [0]	99 (4.0) [0]	119 (14) [0]	89 (8.2) [0]	96 (3.9) [0]	100 (7.3) [0]	97 (5.8) [0]	91 (13) [0]
Full-time	82 (9.7) [−18]	91 (6.3) [−5]	111 (22.3) [+19]	90 (9.1) [−9]	93 (5) [−26]	70 (8.4) [−19]	92 (5.1) [−4]	114 (21.8) [+14]	85 (10.9) [−12]	76 (7) [−15]
Day	93 (11.9) [−7]	102 (14) [+6]	125 (34) [+43]	89 (11.8) [−10]	95 (5) [−24]	84 (5.6) [−5]	102 (4.7) [+6]	113 (17.5) [+13]	98 (8.3) [+1]	93 (7) [+2]
Night	90 (5.4) [−10]	98 (9.4) [+2]	105 (32) [+23]	99 (9.8) [0]	94 (7) [−25]	86 (4.4) [−3]	99 (4.7) [+3]	104 (41) [+4]	96 (7.4) [−1]	91 (5) [0]

Note: Values in square brackets are mean percent differences between loaded and control growth plates, adjusted for the magnitude of the difference observed in the sham operated animals.
Full-time = 24/24 h loading; day = loaded lights-on–lights-off; night = loaded lights-off–lights-on.
Sham = no spring forces.
h_{max} = Hypertrophic chondrocytes final height estimate; LI = BrdU-positive labeling index (proliferative zone).
N_{prolif} = total number of proliferative zone chondrocytes with visible nucleus.

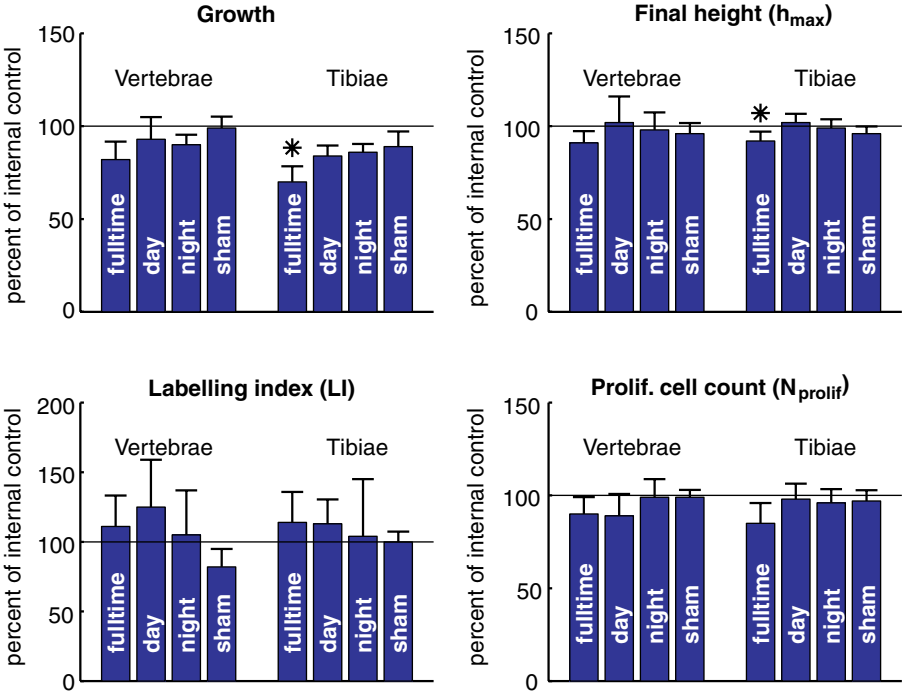

Fig. 3. Bar-graphs of mean values (taken from Table 2) for each group of animals of the measurements of growth, of final chondrocytic height (h_{max}), of the number of proliferative cells N_{prolif}, and of the proliferative cell BrdU-positive labeling index (LI), expressed as percentages of within-animal control values. Error bars represent the standard deviations for each group. ✱ = full-time loaded growth plates differ from other groups ($p < 0.05$).

A significant correlation was observed between growth and final chondrocytic height (h_{max}) at tibial growth plates ($r = 0.64$ for loaded growth plates; $r = 0.53$ for controls). A negative correlation was found between the number of proliferative cells N_{prolif} and the labeling index (LI) ($r = -0.57$ for loaded vertebral growth plates; $r = -0.61$ for control vertebrae, $r = -0.56$ for control tibial growth plates). Percentage differences in growth correlated significantly with percentage differences in N_{prolif} ($r = 0.50$ for vertebrae; $r = 0.49$ for tibiae); with h_{max} ($r = 0.38$ for vertebrae; $r = 0.38$ for tibiae), and with growth plate height ($r = 0.50$ for vertebrae; $r = 0.49$ for tibiae). Based on Eq. (2) it was expected that the percentage change in growth of loaded growth plates would be the sum of the percentage changes in N_{prolif}, LI and h_{max}. However, the percentage change in growth in loaded growth plates was similar to the sum of the percentage changes in final height (h_{max})

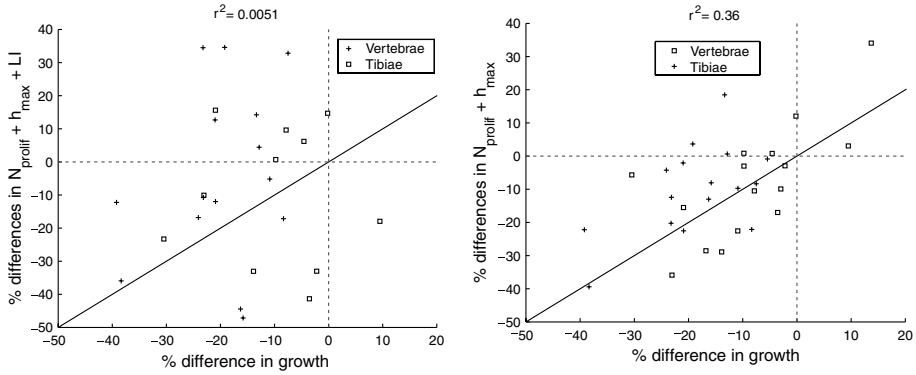

Fig. 4. (Left) *X*-axis: percentage difference (loaded-control) in growth of compressed growth plates. *Y*-axis: sum of the percentage differences in the number of proliferative chondrocytes (N_{prolif}), labeling index (LI) and estimated hypertrophic chondrocytic final height ($h_{\max}$). The correlation coefficient is 0.1. (Right) Same *X*-axis: *Y*-axis: sum of the percentage differences in number of proliferative chondrocytes (N_{prolif}) and in the estimated final cell height ($h_{\max}$). The correlation coefficient is 0.6. The solid line in each case represents the expected equality between the percentage difference in growth and the sum of the percentage changes in histological measures thought to determine growth rate.

and number of proliferative cells (N_{prolif}), but the labeling index (LI) was altered in the opposite sense. This expected relationship was examined by correlation analysis (Fig. 4). The omission of labeling index (LI) in Fig. 4(right) (LI differences were not statistically significant) resulted in the data points lying closer to the expected 1:1 relationship between the percentage change in growth rate and the sum of the percentage changes in the histological measurements that were expected to determine growth rate.

Some data were lost because of technical errors—BrdU data were missing for two animals (one each in the sham and daytime loaded groups) and growth data were missing for one animal in the full-time loaded group.

Discussion

A compressive stress of 0.1 MPa was found to suppress growth of both vertebral and tibial growth plates relative to controls, with the magnitude of this effect being greater in full-time loaded growth plates. As a confounding factor, a reduction in proximal tibial growth was observed in sham operated limbs. Relative to that observed in sham animals, the growth reduction at the two anatomical locations was by a similar proportional amount, and the half-time loading effect on growth approximated half that of full-time loading. There was no evident difference between night-time and day-time loading. This mechanically reduced growth was apparently produced by a combination of reduced numbers of proliferating chondrocytes and lesser chondrocytic height increase in the hypertrophic zone in full-time loaded growth plates, but in those growth plates that were half-time loaded no changes were evident in the hypertrophic zone.

The kinetics of growth plates has been studied previously by analyzing differences between anatomical locations in rats. These analyses show that growth correlated with cell enlargement [3] and with the BrdU labeling index [20]. Here, based on those observations, and from a priori considerations, we expected daily longitudinal growth to equal approximately the product of the number of new cells produced per day and the final chondrocytic height. (This assumes that all new cells complete their differentiation into fully hypertrophied chondrocytes, and that there is no significant thickness of extra-cellular matrix between cells in the growth direction.) Based on this simplified kinetic model, the percentage change in growth was expected to be equal to the sum of the percentage changes in labeling index, number of proliferating cells, and their final height.

The relative estimates of proliferating cell numbers and final cell height appeared to have this additive relationship with growth in the full-time loaded growth plates, but the labeling index demonstrated a non-significant opposite trend. In full-time compressed growth plates the reduction of hypertrophic chondrocytic enlargement together with a reduced count of proliferative chondrocytes explained much of the variation in growth (Fig. 3). In half-time loaded growth plates the smaller growth modulation effect was not consistently associated with any corresponding change in the histological measurements.

The correlation of mechanically altered growth rate with growth plate height was consistent with the idea that growth plate height is an approximate indicator of the product of the number of active cells and their average size, which in turn was thought to correlate with growth rate. Matrix synthesis, that could be expected to have a relationship to chondrocytic enlargement was not measured in this study, based on expectations from the

literature [10,12] that it would not be an accurate indicator of growth rate.

Bone growth is known to vary with the time of day [16], and to be influenced by mechanical loading, but little is known about the mechanical modulation of growth with respect to the time of day. Recently, it was found [22] that elongation of growth plates of lambs is acutely sensitive to whether the limb is weight-bearing. The relative timing of the mechanical stimulus to growth and the growth response has been of considerable interest in the field of orthodontics, where night-time appliances are often employed to displace teeth [11]. Mechanical stimuli can also stimulate growth in the cranial base cartilage [19], and oscillating tensile forces appear to accelerate these processes.

There are few reports of chondrocytic histological changes associated with compressive forces. Alberty et al. [1] reported that compression forces at the rabbit distal femur reduced the numbers of proliferating chondrocytes. The compression was generated by a displacement-controlled apparatus, and probably produced higher stresses than those applied here. These authors did not observe a complementary effect of distraction, although Apte and Kenwright [2] reported that distraction of rabbit proximal tibial growth plate also inhibited cell proliferation.

Several limitations of the present study should be noted. Firstly, the interpretation of the data was complicated by the relatively large sham effect (growth suppression) observed in tibial growth plates, whose cause is not evident. Placement of apparatus was less invasive at the tail than the proximal tibia, where the sham effect was greater. Based on clinical experience of surgical management of fractures [5] most postoperative effects (unloading, increased vascularity, etc.) would be expected to increase not reduce the growth rate. In the experimental design it was assumed that growth and cellular activity had reached a new steady state by the time of euthanasia seven days after installation of the loading apparatus. This time interval was selected based on factors that included the cycle time of rat growth plate chondrocytes, that is reported in the range 30–80 h for growth rates between 400 and 47 μm/day [21].

Timing the intermittent loading to the artificial daylight cycle does not exclude the possibility of other diurnal cycles of response to mechanical stimulus—a full examination of these effects would require groups of animals with loads applied and removed at intermediate time points between the 'lights-on' and 'lights-off' times.

These findings have relevance to the mechanism of progression of skeletal deformity during growth, and its management by braces and other mechanical measures. Application of the present findings to human bone growth should take into consideration the growth rates and the activity levels of humans relative to the rats studied here. Rats are generally considered to be nocturnal animals, but it is not known how the activity levels of the lab animals we studied varied with the time of day. Young rats were selected because of their rapid growth rate.

It should be noted that the findings are specific to the nominal 0.1 MPa compressive stress applied in these experiments. This level of stress was selected because it was thought to be representative of physiological levels of sustained stress that might occur in vivo and as a result of skeletal deformities, but the amount of growth modulation observed was a relatively small proportion of the growth occurring. Robling et al. [15] and Ohashi et al. [13] applied a compressive force of 4, 8.5 or 17 N to rat ulnae, in 10 min steady or cyclic loading daily bouts (compared with approximately 1.7 and 1.0 N applied continuously or diurnally to vertebrae and tibiae in the present study). They reported growth arrest at the higher loading magnitude, and growth suppression on the order of 10% with 4 or 8.5 N loading. Thus the brief (10 min per day) supra-physiological magnitude intermittent loading produced a growth suppression comparable with the unphysiological sustained loading of physiological magnitude that was employed in the present study.

Although endochondral bone growth is influenced by sustained mechanical loading, the level of habitual activity does not apparently alter final skeletal size. Therefore, it appears that intermittent or fluctuating forces do not affect accumulated growth. The 24-h loading cycle investigated here may represent a loading regimen that lies between sustained and intermittent. It produced some modulation of growth, but the effect was small and not clearly associated with any histological changes.

Acknowledgements

This study was supported by NIH R01 AR 46543.

Richard Single suggested the logistic regression relationship to quantify chondrocytic enlargement, and advised on the statistical analyses of data.

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