

= Abstract =

Altered Cellular Kinetics in the Growth Plate according to Alterations in the Weight Bearing

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Purpose: To examine the effects of change in the weight bearing on the growth plate metabolism, a simulated animal model of weightlessness was introduced and the chondrocytes' cellular kinetics were evaluated.

Materials and Methods: Unloading condition on the hind-limb of Sprague-Dawley rats was created by fixing a tail and lifting the hind-limb. Six rats aged 6 weeks old were assigned to each group of unloading, reloading, and control groups of unloading or reloading. Unloading was maintained for three weeks, and then reloading was applied for another one week thereafter. Histomorphometry for the assessment of vertical length of the growth plate, 5-bromo-2'-deoxyuridin (BrdU) immunohistochemistry for cellular kinetics, and biotin nick end labeling TUNEL assay for chondrocytes in the growth plate were performed.

Results: The vertical length of the growth plate and the proliferative potential of chondrocytes were decreased in the unloading group than those of control groups. Inter-group differences were more significant in the proliferative and hypertrophic zones. Reloading increased the length of growth plate and proliferative potential of chondrocytes as evidenced by increase of the ratio of positive BrdU stained cells. However, apoptotic changes in the growth plate were not affected by the alterations of weight bearing.

Conclusion: Alterations in the weight bearing induced changes in the chondrocytic proliferative potential of the growth plate and have no effects on the apoptosis occurred. This may suggest that deprived weight bearing due to various clinical situations hamper normal longitudinal bone growth, and further studies regarding the factors for reversibility of chondrocytic proliferation upon variable mechanical stresses are needed.

Key Words: Cellular kinetics, Growth plate, Changes in weight bearing

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* 2003

(cellular kinetics)
가

(anti-gravity muscle)

가

1.

(lower-limb suspension method)

Nyhan ¹⁵⁾

(osteoblast)

(unloading)

(mineral)

9,13,14,19)

tygon

(Fig. 1).

(reloading)

1

가 (irreversible) 가
가

(tubular bone)
(bone loss)

(remodeling)

formation)

(periosteal bone

(mechanical load)

(angular deformity)

^{3,6)}
(growth plate)

가

가

(histomorphometric)



Fig. 1. Hind-limb suspension method.

2. gram (Fig. 2).
 6 Sprague-Dawley ((zone)
 100 g) 6
 (unloading)
 (reloading) 2)
 -20 5-bromo-2'-deoxyuridin
 3 (unloading group) (BrdU, SIGMA) phosphated buffered
 1 (reloading group) saline (PBS) 25
 (age-matched control group) . mg/kg) . , 2 (100
 가 xylene 10 3
 100, 90, 70% ethanol
 . 3% hydrogen peroxide
 4% paraformaldehyde 2 10% (H₂O₂) peroxidase
 ethylenediaminetetracetic acid(EDTA) 0.4% pepsin 20
 . 2N HCl 30 DNA , 5:1
 4 μm silane coat-
 ing slide (MUTO PURE CHEMICALS) BrdU (SIGMA) 12
 hematoxylin eosin . Avidin-biotin (mouse IgG, extra
 . avidin; SIGMA)
 (immunohistochemistry)
 3. goat serum
 1) PBS . 3, 3'-
 diaminobenzidine (DAB, Vector laborato-
 ries) Mayer's hematoxylin
 (resting zone),
 (proliferation zone) (hyper-
 trophic zone) ImageProPro 150
 μm (250 μm
 (zone)
 3)
 (biotin nick end labeling TUNEL assay)
 Tris-HCl (pH 8.0)
 10 20 proteinase K
 (SIGMA) . PBS
 3% H₂O₂ peroxidase
 PBS .
 DNA



Fig. 2. Histomorphometric measurement of growth plate.

buffer 10 DNase (F. Hoffmann-La Roche) 37 2 30
 Hoffmann-La Roche) 20 dUTP
 DNA . Transferase-mediated deoxyuridin triphosphated (TdT, SIGMA) buffer 15 terminaldeoxytransferase Biotin-16-2'-deoxy-uridine-5'-triphosphate(dUTP, F. Hoffmann-La Roche) 30
 TB buffer 10 가 2% Bovine serum albumin(BSA, SIGMA) 15 blocking
 PBS streptavidin-peroxidase (SIGMA) 30 DAB
 nuclear fast red
 70, 90, 100%
 TUNEL assay

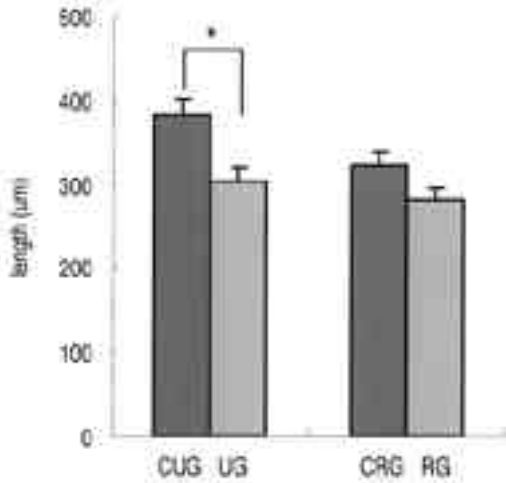


Fig. 3. Total length of the growth plate (*p<0.05). UG: unloading group, CUG: control for unloading, RG: reloading group, CRG: control for reloading group

× 250 µm
 150 µm
 Wilcoxon signed rank test, p=0.05

1.

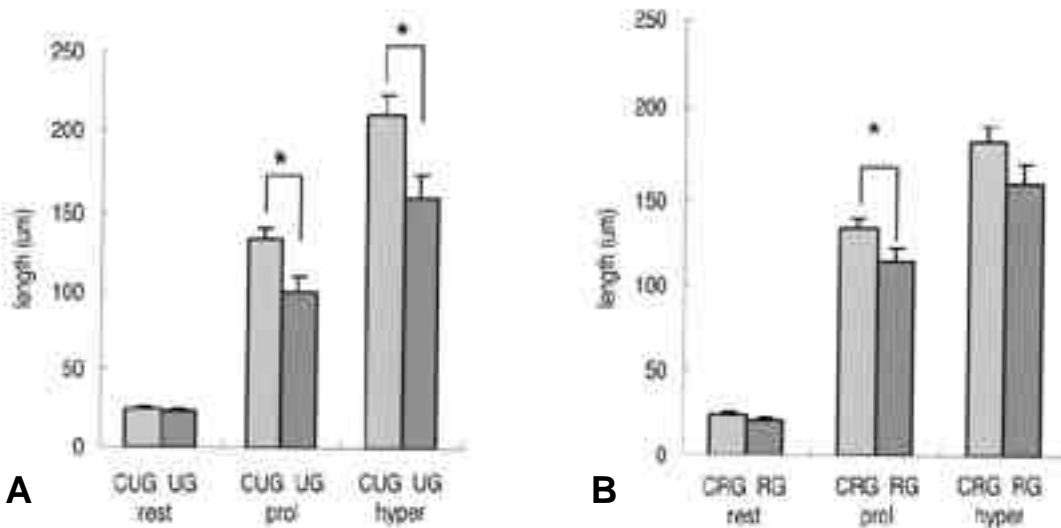


Fig. 4. Length of each zone of the growth plate (*p<0.05). (A) Comparison between unloaded and control group (B) Comparison between reloaded and control group, Rest: resting zone, prol: proliferative zone, hyper: hypertrophic zone

(; $382.9 \pm 11.7 \mu\text{m}$,
 ; $324.5 \pm 13.8 \mu\text{m}$) 가 (Fig. 4).
 $305.3 \pm 14.2 \mu\text{m}$ (79.7%), 281.7
 $\pm 10.3 \mu\text{m}$ (86.8%)
 가 (Fig. 3).

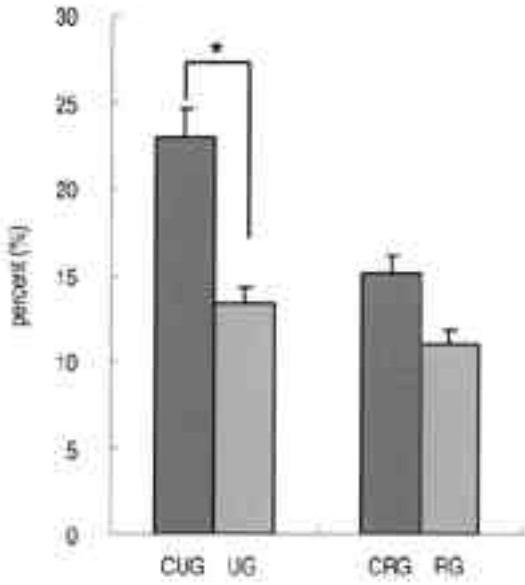


Fig. 6. Comparison of BrdU Immunohistochemistry between groups (*p<0.05).

(zone),
 가 (Fig. 4).

2.

BrdU
 (Fig. 5)
 $22.9 \pm 7.4\%$ $15.1 \pm 4.8\%$

$13.3 \pm 4.1\%$ 57.8% ,
 $11.0 \pm 3.8\%$ 86.8%
 (Fig. 6).

3.

(Fig. 7)
 $31.9 \pm 8.7\%$ $32.9 \pm 9.4\%$

$35.8 \pm 7.2\%$ $36.3 \pm 6.9\%$
 가 (Fig. 8),
 (zone)
 가 (data not shown).

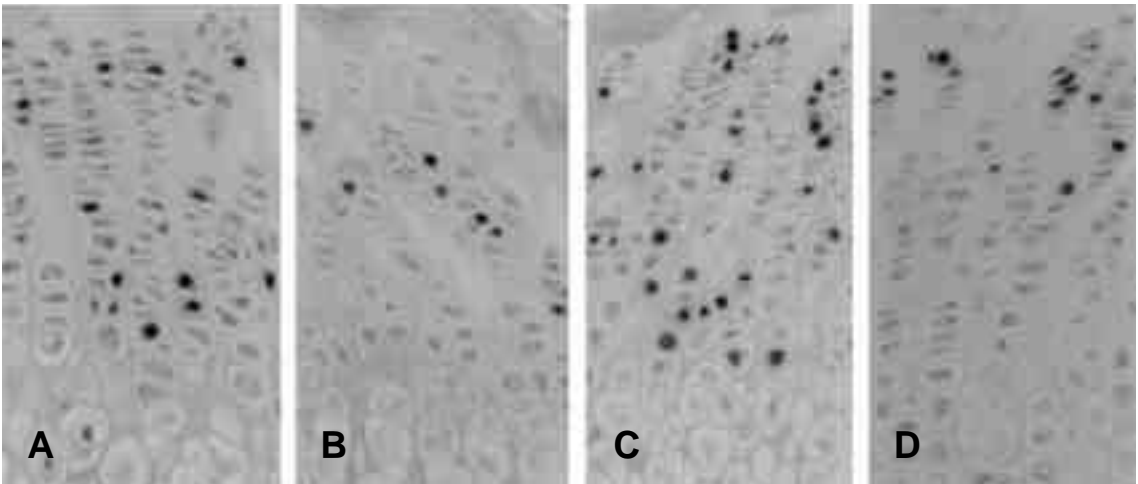


Fig. 5. Findings of BrdU immunohistochemistry (x 100) (A) Control group, (B) Unloading group, (C) Control for reloading group, (D) Reloading group

가 (columnar factors) (local growth pattern) (resting zone), (proliferation zone), (hypertrophic zone) (mineralizing zone) 1,4)

가 (extracellular matrix) 가 (metaphysis) (osteogenic precursor cells), (chondroblast) 가

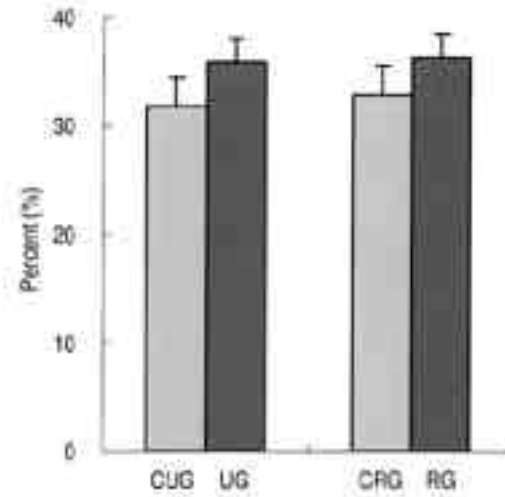


Fig. 8. Comparison of apoptosis between groups.

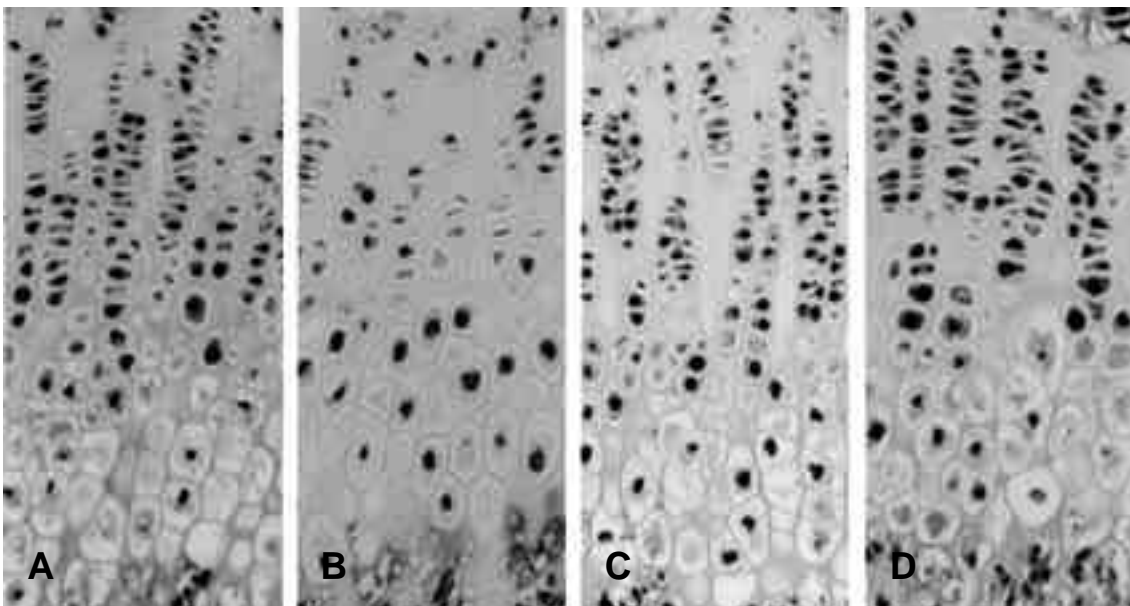


Fig. 7. Apoptosis in the growth plate ($\times 100$, TUNEL assay). (A) Control group, (B) Unloading group, (C) Control for reloading group, (D) Reloading group

가
 가 (reversible)
 ity) 가 가 가
 (irreversible)
 BrdU
 5-bromo-2'-deoxyuridin
 (BrdU)가 S-phase DNA
 thymidine
 3
 가 24 가
 2 anti-BrdU
 5,18,20) 1 가 가
 22.9%
 15.1%

57.8%, 86.8%

가

(lower hypertrophic zone)

TUNEL assay 가 DNA^{7,10,16)}
 DNA
 가
 7)
 34% 가
 가

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