Effects of Growth Hormone on Bone in Mini Rats

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Abstract: Mini rats have been reported to be a Wistar-derived strain of transgenic rats that harbors a rat growth hormone (GH) antisense gene and shows lower plasma GH levels than Wistar rats. Our previous study has revealed that: 1) Mini rats show smaller hind limbs with lower metaphyseal and diaphyseal bone mass than Wistar rats; 2) that the differences of the diaphyseal cortical bone mass and metaphyseal bone mass between Mini rats and Wistar rats are due largely to the differences in the periosteal bone formation and longitudinal growth rate, respectively, and; 3) that the bone turnover in the secondary spongiosa, an area where bone remodeling may occur, does not differ between the two strains. In the present study, 8-week-old male Mini rats were treated intraperitoneally with GH at doses of 0, 2, 6 or 20 IU/kg/time twice daily for 14 days. After the dosage period, we measured the size, bone mineral content, and mineral density of the femur and tibia in each group, and we performed cancellous and cortical bone histomorphometry on the tibia to examine the effects of GH on the bone modeling and remodeling. In the GH-treated groups there were increases in the metaphyseal cancellous bone mass, growth plate width, longitudinal growth rate, and periosteal bone formation accompanied by increases in bone size, mineral content, and mineral density in a dose-related fashion. However, bone histomorphometric parameters related to bone turnover in the secondary spongiosa of the metaphysis, where lamellar bone formation took place, did not differ between the control and GH-treated groups. These findings indicated that GH may not influence bone remodeling in the latter groups, and that GH replacement may not influence the number of osteoclasts involved in bone resorption in Mini rats as a whole. (J Toxicol Pathol 1999; 12: 191~202)

Key words: Mini rat, Growth hormone, Bone histomorphometry, Tartrate-resistant acid phosphatase, Bone remodeling

Introduction

Jcl: Wistar-TgN (ARGHGEN) 1Nts rats (Mini rats) are a strain of Jcl: Wistar rat-derived transgenic animals with reduced plasma growth hormone (GH) levels and growth retardation due to suppression of the expression of the rat GH gene by the presence of an antisense transgene^{1,2}. GH is thought to play an important role in growth, including the growth of bone³⁻⁵. GH is the only hormone among pituitary hormones capable of stimulating longitudinal skeletal growth in a dose-dependent manner³. *In vitro* studies have shown, first, that GH acts directly on osteoblasts^{4,6} and indirectly on bone cells via local insulin-like growth factor I^{4,7}, and second, that it stimulates osteoclastic bone resorption through both direct and indirect actions on osteoclast differentiation and activation of mature osteoclasts^{4,8}. *In vivo* studies have shown that systemic GH administration increases bone mass and histological and biochemical parameters of bone turnover in animals and humans⁹⁻¹¹. In a previous study¹² we compared the bones of Mini rats and Wistar rats at 8 and 22 weeks of age, and found

that the femurs of Mini rats were smaller, had lower mineral densities and were histologically characterized by reductions of metaphyseal and diaphyseal bone mass, compared with those of Wistar rats. For these reasons, Mini rats are thought to be a possible animal model for osteopenia. In another study, we examined and compared hind limbs from these two strains of rats at 10 to 60 weeks of age and found reduced metaphyseal cancellous and diaphyseal cortical bone mass in the Mini rats. Bone histomorphometry on the tibia in this study suggested that the reductions of bone mass in the metaphysis and diaphysis in Mini rats might have been attributable to the lower longitudinal growth rate and periosteal bone formation rate, respectively, and no difference was found in the bone turnover in the secondary spongiosa, an area where bone remodeling may occur. In short, it was suggested that the reduced bone mass in Mini rats may be attributable to the reduced bone formation¹³. On the other hand, since it has also been reported that GH stimulates bone turnover and bone formation, in the present study we administered GH at three dose levels to Mini rats, measured the bone size, mineral content, and mineral density of the bone removed, and performed bone histomorphometry in order to investigate the effects of GH on bone, including bone modeling and remodeling.

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Materials and Methods

Experimental Animals

Male Mini rats^{1,2} were purchased from Nisseiken Co., Ltd. (Tokyo, Japan) and used at 8 weeks of age. This age was determined based on the results of a preliminary study in which male Mini rats of 8 to 58 weeks of age were treated intraperitoneally with porcine GH at a dose of 2 IU/Kg/time twice daily for 14 consecutive days (Unpublished data, Teranishi M *et al.*). The rats were housed in groups of 5 per cage in a stainless cage with sawdust for bedding in an air-conditioned room (temperature of $23 \pm 1^\circ\text{C}$, relative humidity of $55 \pm 10\%$) with a 12-hr-light, 12-hr-dark cycle. They were given an irradiated pelleted diet (F-2, Funabashi Farm, Chiba) and water *ad libitum*. All animal manipulations were performed according to our institutional animal care and use standards.

Growth Hormone, Methods of Administration, and Group Composition

Porcine pituitaries-derived somatotropin (growth hormone) (Sigma Chemical Co., St. Louis) was dissolved in Tris-buffered physiological saline (pH 7.6) containing 1.6% glycerol, 0.02% sodium azide, and 0.01% bovine serum albumin to obtain a GH concentration of 2 IU/ml, and the prepared solution was stored in a refrigerator until use within 2–3 days. Based on the results of the preliminary study (Unpublished data, Teranishi M *et al.*), the GH-treated animals were administered intraperitoneally with GH at dose levels of 2, 6, and 20 IU/kg/time (L-GH, M-GH, and H-GH groups, respectively) twice daily (9:00, 16:00) for 14 consecutive days. As a control group, Mini rats were treated intraperitoneally with vehicle in a volume of 1 ml/kg in the same manner as the GH-treated groups. Each group was composed of 5 rats.

Fluorochrome Labeling of Bones

Calcein (10 mg/kg; Dojindo Laboratories, Kumamoto) and oxytetracycline (20 mg/kg; Showa Yakuhin Kako Co., Ltd., Tokyo) were used as fluorochromes and administered subcutaneously. The labeling schedules were as follows: 1–1–1–1 (i.e., Calcein 4 days before autopsy and oxytetracycline 2 days before autopsy).

Body Weight and Organ Weight

The animals were weighed twice weekly during the treatment period and the body weight gains were calculated. The brain, heart, liver, kidneys, spleen, thymus, pituitary, adrenals, and testes were weighed, and the weights of these organs relative to the final body weight were calculated.

Hematological and Blood Chemical Examinations

On each autopsy day, Mini rats were anesthetized with ether and then exsanguinated after blood collection from the aorta for hematological (red blood cell, RBC; white blood cell, WBC; platelet, PLT) and serum biochemical analyses (alkaline phosphatase, ALP; calcium, Ca; inorganic phosphorus, IP).

Pathological Examinations

At autopsy, bones of hind limbs (femurs and tibiae) were removed and freed from the soft tissues. Bone histomorphometric analysis was performed after gross observa-

tion of the bones and measurement to determine bone size, mineral content, and mineral density. Left femurs and tibiae were immersed and fixed in 70% ethanol, their sizes (the lengths of the femur and tibia, and midshaft diameters of the femur) were measured with a degimatic caliper (Mitutoyo Corporation, Tokyo), and their mineral contents and mineral densities were measured, either as total values or as separate values for proximal, shaft, and distal portions, with a DI-CHROMA SCAN DCS-600 (Aloka Co., Ltd., Tokyo) by single photon absorptiometry. In addition, for assessment of bone histomorphometry, left tibiae were fixed, stained with Villanueva bone stain, dehydrated, embedded in methyl methacrylate, and cut into a 7- μm -thick undecalcified frontal section of the proximal tibia and a 15- μm -thick undecalcified cross-section of the tibial shaft 3-mm-proximal to the tibiofibular junction.

Right femurs and tibiae were immersed and fixed in 0.1 M cacodylate buffer containing 4% paraformaldehyde at 4°C for one week, decalcified properly in 10% ethylenediaminetetraacetic acid (EDTA) at 4°C , and embedded in soft paraffin with a low solidification point of $42\text{--}44^\circ\text{C}$ (Merck, Darmstadt, Germany) for enzyme histochemistry¹⁴. From these paraffin blocks, 4- μm -thick longitudinal (sagittal) sections of the distal femur and proximal tibia and 4- μm -thick cross-sections of the diaphyseal femur and diaphyseal tibia were initially processed and stained with hematoxylin and eosin (H & E), and then additionally stained histochemically for tartrate-resistant acid phosphatase (TRAP)¹⁵, a marker enzyme of osteoclast function¹⁶. The prepared histological slides were read with a light microscope, and the bone histomorphometry procedures described below were performed on the tibia.

Bone Histomorphometry

I. Undecalcified histological slides of the tibia

Undecalcified frontal sections (5 animals/group) of the left proximal tibia were used for analysis of the status of longitudinal growth in the growth plate and bone formation/resorption in the metaphyseal trabeculae (secondary spongiosa). Undecalcified cross sections (5 animals/group) of the tibial shaft were used for analysis of the status of bone formation in the cortex. Bone histomorphometry was performed using a transmitted and epifluorescent light microscope linked to a computer-assisted image analyzer (Osteoplan II; Carl Zeiss, Germany). Histomorphometric parameters were obtained by tracing relevant features with a cursor on the videoscreen image.

For the growth plate of the proximal tibia, we obtained the growth plate width and longitudinal growth rate¹⁷. For the cancellous bone of the proximal tibia, we measured the secondary spongiosa of the metaphysis as follows: 1) an area ranging 1,760 μm distally from the point 880 μm distal to the growth plate-metaphyseal junction (GPMJ); and, 2) an area ranging 1,320 μm distally from the estimated endochondral ossification front at the start of treatment calculated from the relative longitudinal growth rate. In these two areas double layers of fluorescent labels were definitely seen in the trabeculae along the bone surface, indicating lamellar, not woven, bone formation related to bone remodeling¹⁸. These two areas were set maximal so as not to include the cortical rim. Histomorphometric parameters measured and calculated for the cancellous bone¹⁹ included the following: as static parameters, bone volume (BV/TV) (%), osteoid volume (OV/BV) (%), osteoid surface (OS/BS) (%), osteoid

thickness (O.Th) (μm), osteoblast surface (Ob.S/BS) (%), eroded surface (ES/BS) (%), osteoclast surface (Oc.S/BS) (%), osteoclast number/tissue area (N.Oc/T.Ar) (/mm²), osteoclast number/bone area (N.Oc/B.Ar) (/mm²), osteoclast number/bone perimeter (N.Oc/B.Pm) (/100 mm); as architectural parameters¹⁹, trabecular thickness (Tb.Th) (μm), trabecular number (Tb.N) (/mm), trabecular separation (Tb.Sp) (μm); as dynamic parameters¹⁹, mineral apposition rate (MAR) ($\mu\text{m}/\text{day}$), adjusted apposition rate (Aj.AR) ($\mu\text{m}/\text{day}$), double-labeled surface (dLS/BS) (%), mineralizing surface (MS/BS) (%), bone formation rate/tissue volume (BFR/TV) (mm³/cm³/year), bone formation rate/bone surface (BFR/BS) (mm³/cm²/year), mineralization lag time (Mlt) (days), osteoid maturation time (Omt) (days).

Histomorphometric parameters measured and calculated for the cortical bone included the following: as static parameters^{17,19}, total tissue area (T.Ar) (mm²), cortical bone area (Ct.Ar) (mm²), marrow area (Ma.Ar) (mm²), periosteal perimeter (Ps.Pm) (mm), endocortical perimeter (Ec.Pm); as dynamic parameters^{17,19}, periosteal mineral apposition rate (Ps.MAR) ($\mu\text{m}/\text{day}$), periosteal mineralizing surface (Ps.MS/BS) (%), periosteal bone formation rate (Ps.BFR/BS) (mm³/cm²/year), endocortical mineral apposition rate (Ec.MAR) ($\mu\text{m}/\text{day}$), endocortical mineralizing surface (Ec.MS/BS) (%), endocortical bone formation rate (Ec.BFR/BS) (mm³/cm²/year).

II. Decalcified histochemical slides of the femur

Decalcified longitudinal sections (5 animals/group) of the right distal femur stained histochemically for TRAP were used for analysis of the number of TRAP-positive cells related to bone resorption. These measurements included 0.58 mm² areas of the GPMJ/primary spongiosa, the secondary spongiosa between 880 μm and 1,320 μm distal to the GPMJ, and the secondary spongiosa between 1,760 μm and 2,200 μm distal to the GPMJ. The TRAP-positive cell counts were expressed as the numbers of mononuclear TRAP-positive cells, multinuclear TRAP-positive cells, and total (mononuclear+multinuclear) TRAP-positive cells in each area.

Statistical Analysis

All data are expressed as the mean $\pm$ S.D. in the tables and figures. Statistical analysis of mean values and standard

deviations of body weight, body weight gain, organ weight, hematological and blood chemical parameters, bone size, bone mineral content and density, and bone histomorphometric parameters was performed between the control and GH-treated groups with Dunnett's multiple comparison test. Statistical significance was assumed at $p < 0.05$.

Results

Body Weight and Organ Weight

As shown in Fig. 1 and Table 1, body weight in the GH-treated groups tended to be significantly greater than that in the control group, and the degree of difference tended to be dose-related. Compared with the control group, body weights in the H-GH and M-GH groups showed significantly higher values from day 10 and on day 14, respectively.

The absolute and relative weights of the spleen, liver, and adrenals tended to be greater in the GH-treated groups than in the control group. Compared with the control group, significant differences were observed in the relative

Fig. 1. Body weight changes in Mini rats treated intraperitoneally with GH.

Values are expressed as mean. ($n=5$) *Significantly different from control ($p < 0.05$). Control, treated with vehicle; L-GH, treated with 2 IU/kg/time; M-GH, treated with 6 IU/kg/time; H-GH, treated with 20 IU/kg/time.

Table 1. Body Weight and Relative Organ Weight in Mini Rats Treated Intraperitoneally with GH

Group	Final Bw (g)	BW Gain (g)	Brain (g%)	Heart (g%)	Liver (g%)	Kineny (R) (g%)	Kidney (L) (g%)	Spleen (g%)	Thymus (g%)	Pituitary (mg%)	Adrenal (mg%)	Testis (R) (g%)	Testis (L) (g%)
Control	155.8 ± 11.4	23.3 ± 4.3	1.04 ± 0.07	0.35 ± 0.01	3.41 ± 0.05	0.28 ± 0.02	0.29 ± 0.02	0.21 ± 0.00	0.10 ± 0.00	1.8 ± 0.3	15.3 ± 1.9	0.63 ± 0.04	0.63 ± 0.04
L-GH	161.5 ± 3.5	29.8* ± 2.9	1.02 ± 0.02	0.33 ± 0.02	3.49 ± 0.16	0.29 ± 0.01	0.28 ± 0.02	0.24* ± 0.02	0.11 ± 0.01	1.7 ± 0.3	18.8 ± 2.6	0.60 ± 0.02	0.62 ± 0.03
M-GH	168.5* ± 6.4	36.2* ± 1.1	0.96* ± 0.03	0.33 ± 0.01	3.61 ± 0.18	0.28 ± 0.01	0.27 ± 0.01	0.24* ± 0.00	0.09 ± 0.01	1.9 ± 0.4	18.6 ± 3.2	0.60 ± 0.04	0.61 ± 0.03
H-GH	177.9* ± 5.4	45.3* ± 2.9	0.92* ± 0.03	0.32* ± 0.02	3.76* ± 0.09	0.29 ± 0.02	0.29 ± 0.02	0.26* ± 0.01	0.08* ± 0.00	2.0 ± 0.3	21.0* ± 1.5	0.55* ± 0.02	0.56* ± 0.03

Values are expressed as mean $\pm$ S.D. ($n=5$) ND, not determined.

*Significantly different from control ($p < 0.05$).

Control, treated with vehicle; L-GH, treated with 2 IU/kg/time; M-GH, treated with 6 IU/kg/time; H-GH, treated with 20 IU/kg/time.

BW, body weight; R, right; L, left.

Table 2. Hematological and Blood Chemical Analysis in Mini Rats Treated Intraperitoneally with GH

Group	RBC ($\times 10^6/\text{mm}^3$)	WBC ($\times 10^3/\text{mm}^3$)	PLT ($\times 10^3/\text{mm}^3$)	ALP (U/l)	Ca (mg/dl)	IP (mg/dl)
Control	7.70 ± 0.19	6.19 ± 1.24	873.0 ± 44.0	916.2 ± 45.8	10.0 ± 0.2	7.4 ± 0.4
L-GH	7.56 ± 0.10	6.08 ± 0.39	899.5 ± 35.6	868.3 ± 48.7	10.0 ± 0.2	8.1 ± 0.6
M-GH	7.51 ± 0.20	5.90 ± 0.97	960.0* ± 47.7	989.2 ± 85.9	10.0 ± 0.2	8.1 ± 0.4
H-GH	7.61 ± 0.9	6.85 ± 0.96	1,070.6* ± 45.7	985.4 ± 32.1	10.4 ± 0.8	9.0* ± 0.6

Values are expressed as mean $\pm$ S.D. ($n=5$)

*Significantly different from control ($p < 0.05$).

Control, treated with vehicle; L-GH treated with 2 IU/kg/time; M-GH, treated with 6 IU/kg/time; H-GH, treated with 20 IU/kg/time.

RBC, red blood cell; WBC, white blood cell; PLT, platelet; ALP, alkaline phosphatase; Ca, Calcium; IP, inorganic phosphorus.

weights of the spleen of all GH-treated groups, and the relative weights of the liver and adrenals of the H-GH groups. The relative weights of the kidneys in the GH-treated groups were not significantly different from those in the control group, but the absolute weights of the kidneys were significantly greater in the H-GH group (data not shown). The absolute weights of the brain, heart, thymus, and testes in the GH-treated groups were not significantly different from those in the control group (data not shown), but significantly lower values were observed in the relative weights of the brain in the M-GH and H-GH groups, and the heart, thymus, and testes in the H-GH group.

Hematological and Blood Chemical Examinations

As shown in **Table 2**, hematologically, PLT count was significantly higher in the M-GH and H-GH groups. WBC count tended to be higher, although not significantly, in the H-GH group. In blood chemical examinations, IP was significantly higher in the H-GH group. IP tended to be higher, although not significantly, in the L-GH and M-GH

groups, and ALP tended to be higher, although not significantly, in the M-GH and H-GH groups.

Bone Size of Femur and Tibia

As shown in **Table 3**, the length of the femur was significantly greater in the M-GH and H-GH groups, and the length of the tibia was significantly greater in the H-GH group.

Bone Mineral Content and Mineral Density of Femur and Tibia

As shown in **Table 4**, bone mineral content of the femur in the H-GH group was significantly higher overall and in separate proximal, diaphyseal, and distal portions. Bone mineral content of the tibia in the H-GH group was significantly higher overall and in the proximal portion.

Bone mineral density of the femur was significantly higher overall and in the diaphyseal and distal portions in the H-GH group, in the diaphyseal portion in the M-GH group, and overall and in the proximal portion in the L-GH group. Bone mineral density of the tibia was significantly higher overall and in the proximal portion in the H-GH group.

Histopathology

Histopathologically (H & E histological slides), the GH-treated groups showed increases in the growth plate width of the distal femur and proximal tibia, and increases in the amount of metaphyseal cancellous bone mass (especially, the primary spongiosa). The proportion of the width of the resting zone in the growth plate was seemingly decreased in the GH-treated groups, whereas the proportions of the proliferative and hypertrophic zones and the numbers of chondrocytes were all increased (Fig. 2). Cross-sections of the diaphyseal femur and tibia did not show any differences in the amounts of cortical bone mass and bone marrow areas between the control and GH-treated groups. In the bone marrow, hematopoiesis was increased in the GH-treated groups, being especially prominent in the H-GH group.

Bone Histomorphometry

I. Undecalcified histological slides of the tibia

The growth plate width and longitudinal growth rate of the proximal tibia are shown in Fig. 3. **Table 5** shows static parameters of cancellous bone in the metaphysis of the

Table 3. Bone Size of Femur and Tibia in Mini Rats Treated Intraperitoneally with GH

Group	Femur (mm)			Tibia (mm)
	Length	Midshaft Diameter		Length
		Maximum	Minimum	
Control	28.82 ± 0.40	2.84 ± 0.08	2.46 ± 0.05	33.85 ± 0.36
L-GH	29.24 ± 0.20	2.85 ± 0.08	2.48 ± 0.04	33.78 ± 0.10
M-GH	29.49* ± 0.24	2.90 ± 0.12	2.47 ± 0.03	34.07 ± 0.21
H-GH	29.83* ± 0.27	2.98 ± 0.12	2.50 ± 0.06	34.66* ± 0.02

Values are expressed as mean $\pm$ S.D. ($n=5$)

*Significantly different from control ($p < 0.05$).

Control, treated with vehicle; L-GH, treated with 2 IU/kg/time; M-GH, treated with 6 IU/kg/time; H-GH, treated with 20 IU/kg/time.

Maximum, medial-lateral; Minimum, anterior-posterior.

Table 4. Bone Mineral Content and Bone Mineral Density of Femur and Tibia in Mini Rats Treated Intraperitoneally with GH

Group	Femur					Tibia				
	Bone Mineral Content (mg)			Bone Mineral Density (mg/cm ²)		Bone Mineral Content (mg)			Bone Mineral Density (mg/cm ²)	
	Total	Proximal	Shaft	Distal	Total	Total	Proximal	Shaft	Distal	Total
Control	133.8 ±6.5	46.2 ±2.8	33.4 ±1.7	54.2 ±2.4	100.7 ±3.3	100.7 ±3.3	95.9 ±4.8	90.5 ±2.5	113.4 ±3.3	80.6 ±1.9
L-GH	135.1 ±5.8	47.8 ±2.3	33.3 ±1.2	54.1 ±3.0	105.2* ±2.9	100.7 ±3.8	105.5* ±4.4	91.9 ±2.1	115.3 ±2.9	81.8 ±1.8
M-GH	138.4 ±3.8	48.3 ±1.6	34.9 ±0.9	55.2 ±2.5	104.1 ±2.3	104.3 ±6.4	101.0 ±4.8	94.0* ±1.4	115.0 ±2.2	82.5 ±2.0
H-GH	149.5* ±6.8	50.8* ±1.8	36.6* ±1.8	62.1* ±3.4	107.1* ±2.2	108.3* ±4.5	101.3 ±3.8	94.6* ±2.2	122.4* ±2.9	84.2* ±2.5

Values are expressed as mean ± S.D. (*n* = 5) *Significantly different from control (*p* < 0.05). Control, treated with vehicle; L-GH, treated with 2 IU/kg/time; M-GH, treated with 6 IU/kg/time; H-GH, treated with 20 IU/kg/time.

Table 5. Static Cancellous Bone Histomorphometry of Proximal Tibial Metaphysis in Mini Rats Treated Intraperitoneally with GH (unadjusted to the longitudinal growth rate)

Group	BV/TV (%)	OV/BV (%)	OS/BS (%)	O. Th (μm)	Ob. S/BS (%)	ES/BS (%)	N. Oc /B. Pm (/100 mm)	N. Oc /B. Ar (/mm ²)	N. Oc /T. Ar (/mm ²)	Oc. S/BS (%)	Tb. Th (μm)	Tb. N (/mm)	Tb. Sp (μm)
Control	7.5 ±2.9	7.2 ±1.9	46.6 ±3.1	2.5 ±0.3	32.7 ±3.3	40.3 ±6.9	396.4 ±78.5	200.0 ±45.9	14.7 ±6.5	27.5 ±3.7	33.7 ±3.8	4.4 ±0.6	211.8 ±40.1
L-GH	8.3 ±2.0	7.2 ±0.8	46.3 ±4.1	2.7 ±0.3	31.0 ±7.9	43.7 ±3.7	387.4 ±59.1	189.9 ±39.0	15.6 ±5.0	28.9 ±4.6	34.4 ±3.3	4.6 ±0.6	196.6 ±29.5
M-GH	10.8 ±3.6	6.5 ±1.5	45.7 ±2.7	2.4 ±0.2	33.0 ±2.2	43.6 ±1.5	417.4 ±98.0	208.3 ±68.8	20.7 ±2.0	28.4 ±4.0	34.2 ±3.5	5.2 ±0.6	164.9 ±27.6
H-GH	13.7* ±3.1	4.7* ±0.7	41.0 ±5.7	2.2 ±0.3	27.9 ±5.3	46.0 ±5.3	424.7 ±77.2	189.9 ±61.8	24.7 ±4.2	27.7 ±6.1	38.7 ±6.1	5.3 ±0.5	152.4* ±14.6

Values are expressed as mean ± S.D. (*n* = 5) *Significantly different from control (*p* < 0.05). Control, treated with vehicle; L-GH, treated with 2 IU/kg/time; M-GH, treated with 6 IU/kg/time; H-GH, treated with 20 IU/kg/time. BV/TV, bone volume/tissue volume; OV/BV, osteoid volume/bone volume; OS/BS, osteoid surface/bone surface; O. Th, osteoid thickness; Ob. S/BS, osteoblast surface/bone surface; ES/BS, eroded surface/bone surface; N. Oc/B. Pm, osteoclast number/bone perimeter; N. Oc/T. Ar, osteoclast number/tissue area; N. Oc/B. Ar, osteoclast number/bone area; Oc. S/BS, osteoclast surface/bone surface; Tb. Th, trabecular thickness; Tb. N, trabecular number; Tb. Sp, trabecular separation.

Table 6. Static Cancellous Bone Histomorphometry of Proximal Tibial Metaphysis in Mini Rats Treated Intraperitoneally with GH (adjusted to the longitudinal growth rate)

Group	BV/TV (%)	OV/BV (%)	OS/BS (%)	O. Th (μm)	Ob. S/BS (%)	ES/BS (%)	N. Oc /B. Pm (/100 mm)	N. Oc /B. Ar (/mm ²)	N. Oc /T. Ar (/mm ²)	Oc. S/BS (%)	Tb. Th (μm)	Tb. N (/mm)	Tb. Sp (μm)
Control	8.0 ±2.2	6.3 ±1.4	43.6 ±2.6	2.4 ±0.4	34.7 ±3.6	44.4 ±2.3	400.3 ±61.4	206.3 ±43.4	16.1 ±3.8	30.1 ±2.9	32.7 ±2.8	4.7 ±0.4	251.9 ±135.1
L-GH	6.9 ±2.1	6.2 ±1.7	41.8 ±6.8	2.6 ±0.6	28.9 ±7.9	50.0 ±7.1	388.2 ±52.5	186.2 ±15.5	12.7 ±3.6	28.4 ±4.9	34.8 ±4.1	4.1 ±0.5	226.7 ±34.6
M-GH	8.9 ±2.9	6.7 ±1.4	48.7 ±4.8	2.4 ±0.5	34.4 ±6.0	42.5 ±7.1	390.4 ±78.4	182.2 ±29.7	15.7 ±3.5	26.3 ±7.5	35.8 ±5.2	4.6 ±0.9	199.9 ±54.7
H-GH	6.1 ±1.3	5.4 ±1.8	38.4 ±6.1	2.3 ±0.6	31.3 ±6.1	47.9 ±4.0	472.9 ±129.3	251.5 ±87.5	15.2 ±5.2	35.7 ±6.8	32.1 ±3.7	4.2 ±0.6	223.0 ±34.8

Values are expressed as mean ± S.D. (*n* = 5) *Significantly different from control (*p* < 0.05). Control, treated with vehicle; L-GH, treated with 2 IU/kg/time; M-GH, treated with 6 IU/kg/time; H-GH, treated with 20 IU/kg/time. BV/TV, bone volume/tissue volume; OV/BV, osteoid volume/bone volume; OS/BS, osteoid surface/bone surface; O. Th, osteoid thickness; Ob. S/BS, osteoblast surface/bone surface; ES/BS, eroded surface/bone surface; N. Oc/B. Pm, osteoclast number/bone perimeter; N. Oc/T. Ar, osteoclast number/tissue area; N. Oc/B. Ar, osteoclast number/bone area; Oc. S/BS, osteoclast surface/bone surface; Tb. Th, trabecular thickness; Tb. N, trabecular number; Tb. Sp, trabecular separation.

Fig. 2. Light photomicrographs of the distal femoral growth plate and metaphysis from a control Mini rat (A) and an H-GH Mini rat (B).

Treatment with GH tends to increase the growth plate width and metaphyseal cancellous bone mass of the distal femur, especially in the primary spongiosa. The width of the resting zone in the growth plate is decreased, and in turn, the widths of the proliferative and hypertrophic zones shows increases together with increases in the numbers of chondrocytes in the latter two zones. H-GH, treated with 20 IU/kg/time. H & E stain. Bar = 1 mm.

proximal tibia when the proximal point from the GPMJ was set in the same manner in all groups, and **Table 6** shows the same parameters when this point was adjusted to the respective longitudinal bone growth rates. Similarly, **Tables 7** and **8** show dynamic parameters of cancellous bone in the metaphysis of the proximal tibia under the same conditions, respectively. Static and dynamic parameters of cortical bone in the tibial shaft are shown in **Table 9**. In addition, typical photomicrographs for frontal sections of the proximal tibia and cross-sections of the tibial shaft are shown in Figs. 4 and 5.

As shown in Fig. 3, the width of the growth plate and the longitudinal growth rate in the proximal tibia were increased in a dose-related manner. Compared with the control group, the width of the growth plate was significantly greater in the M-GH and H-GH groups, and the longitudinal growth rate was significantly greater in all GH-treated groups.

As shown in **Table 5**, when we measured the area of the secondary spongiosa in which the proximal point from the GPMJ was set in the same manner in all groups (880 μm distal to the GPMJ), BV/TV was significantly greater in the H-GH group, and OV/BV and Tb.Sp were significantly lower in the H-GH group. BV/TV, OV/BV, and Tb.Sp all tended to increase or decrease in a dose-related manner. On the other hand, as shown in **Table 6**, when the proximal point from the GPMJ was adjusted to the respective longitudinal bone growth rates (control, ca. 900 μm ; L-GH, ca. 1,100 μm ; M-GH, ca. 1,230 μm ; H-GH, 1,520 μm), there were no significant differences in the static histomorphometric parameters of cancellous bone, including the above-mentioned parameters between the control and GH-treated groups.

As shown in **Tables 8** and **9**, there were no significant differences in the dynamic histomorphometric parameters of cancellous bone of the secondary spongiosa between the control and GH-treated groups, regardless of whether the measured area was adjusted to the respective longitudinal growth rates.

As shown in **Table 9**, there were no significant differences in the static histomorphometric parameters of tibial diaphyseal cortical bone between the control and GH-treated groups. As for dynamic histomorphometric parameters, Ps.MAR in the M-GH and H-GH groups, Ps.MS/BS in the L-GH and H-GH groups, and Ps.BFR/BS in the H-GH group were all significantly higher than the corresponding parameters in the control group. On the other hand, there

Fig. 3. Growth plate width (A) and longitudinal growth rate (B) of the proximal tibia in Mini rats treated intraperitoneally with GH.

Values are expressed as mean $\pm$ S.D. ($n=5$) *Significantly different from control ($p<0.05$). Control, treated with vehicle; L-GH, treated with 2 IU/kg/time; M-GH, treated with 6 IU/kg/time; H-GH, treated with 20 IU/kg/time.

Fig. 4. Fluorescent photomicrograph of the proximal tibial growth plate and metaphysis from a control Mini rat (A) and an H-GH Mini rat (B).

Treatment with GH increases the fluorescent label interval along the longitudinal axis in the proximal metaphysis in a dose-related manner. This increase accompanies the dose-related increases in the growth plate width and cancellous bone mass, especially the primary spongiosa. On the other hand, there is no apparent difference in the fluorescent labels along the bone surface of the secondary spongiosa between the control and GH-treated rats. H-GH, treated with 20 IU/kg/time. Villanueva bone stain. Bar = 200 μ m.

were no differences in the endocortical counterparts (Ec.MAR, Ec.MS/BS, Ec.BFR/BS) between the control and GH-treated groups, and there was no consistent tendency in changes of the endocortical parameters appearing as a result of the GH treatment.

As shown in Fig. 4, intervals between the first fluorescent label and the second fluorescent label along the longitudinal axis in the tibial proximal metaphysis increased in a dose-related manner, together with the growth plate width and the amount of cancellous bone mass (especially, primary spongiosa). On the other hand, there were no clear differences in the fluorescent labels along the bone surface of the secondary spongiosa between the control and GH-treated groups. As shown in Fig. 5, the total length of the fluorescent labels

Fig. 5. Fluorescent photomicrograph of the tibial cortex from a control Mini rat (A) and an H-GH Mini rat (B).

Treatment with GH increases the total length of fluorescent labels along the periosteum of the diaphyseal cortex in the GH-treated rat in a dose-related manner, whereas treatment with GH may not influence the total length of fluorescent labels along the endosteum, as shown by bone histomorphometry. H-GH, treated with 20 IU/kg/time. Villanueva bone stain. Bar = 200 μ m.

along the diaphyseal periosteum in the GH-treated groups seemed to increase in a dose-related manner, whereas the counterpart along the diaphyseal endosteum did not seem to be related to the GH dose. These findings were consistent with the above results on bone histomorphometry.

II. Decalcified histochemical slides of the femur

The results of measurement of TRAP-positive cells in the longitudinal sections of the distal femur are shown in **Tables 10 to 12**. In the area of the GPMJ/primary spongiosa (**Table 10**), there were no significant differences in the TRAP-positive cell numbers (mononuclear, multinuclear, and total) per tissue area between the control and GH-treated groups. In the area of the secondary spongiosa between 880 μ m and 1,320 μ m distal to the GPMJ (**Table 11**), there were no significant differences in TRAP-positive cell numbers (mononuclear, multinuclear, and total) per tissue area, per bone volume, and per bone perimeter between the control and GH-treated groups. In the area of the secondary spon-

Table 7. Dynamic Cancellous Bone Histomorphometry of Proximal Tibial Metaphysis in Mini Rats Treated Intraperitoneally with GH (unadjusted to the longitudinal growth rate)

Group	MAR ($\mu\text{m}/\text{day}$)	Aj. AR ($\mu\text{m}/\text{day}$)	dLS/BS (%)	MS/BS (%)	BFR/TV ($\text{mm}^3/\text{cm}^3/\text{year}$)	BFR/BS ($\text{mm}^3/\text{cm}^2/\text{year}$)	Mlt (days)	Omt (days)
Control	2.42 ± 0.26	0.90 ± 0.25	11.5 ± 2.4	16.9 ± 2.5	681.2 ± 308.9	15.1 ± 3.6	3.7 ± 1.6	1.3 ± 0.3
L-GH	2.59 ± 0.39	0.90 ± 0.30	12.0 ± 4.4	16.2 ± 5.1	722.9 ± 271.8	15.4 ± 6.2	4.0 ± 1.9	1.3 ± 0.2
M-GH	2.45 ± 0.32	0.89 ± 0.37	11.4 ± 3.4	16.0 ± 4.9	847.4 ± 189.5	14.7 ± 5.8	3.9 ± 2.1	1.2 ± 0.2
H-GH	2.54 ± 0.37	0.68 ± 0.13	7.0 ± 2.8	11.3 ± 3.7	716.6 ± 193.9	10.3 ± 2.9	4.0 ± 0.7	1.0 ± 0.1

Values are expressed as mean $\pm$ S.D. ($n=5$)

*Significantly different from control ($p<0.05$).

Control, treated with vehicle; L-GH, treated with 2 IU/kg/time; M-GH, treated with 6 IU/kg/time; H-GH, treated with 20 IU/kg/time.

MAR, mineral apposition rate; Aj. AR, adjusted apposition rate; dLS/BS, double-labeled surface/bone surface; MS/BS, mineralizing surface/bone surface; BFR/TV, bone formation rate/tissue volume; BFR/BS, bone formation rate/bone surface; Mlt, mineralization lag time; Omt, osteoid maturation time.

Table 8. Dynamic Cancellous Bone Histomorphometry of Proximal Tibial Metaphysis in Mini Rats Treated Intraperitoneally with GH (adjusted to the longitudinal growth rate)

Group	MAR ($\mu\text{m}/\text{day}$)	Aj. AR ($\mu\text{m}/\text{day}$)	dLS/BS (%)	MS/BS (%)	BFR/TV ($\text{mm}^3/\text{cm}^3/\text{year}$)	BFR/BS ($\text{mm}^3/\text{cm}^2/\text{year}$)	Mlt (days)	Omt (days)
Control	2.45 ± 0.26	1.28 ± 0.33	16.1 ± 2.2	22.3 ± 2.9	984.9 ± 325.3	20.1 ± 4.2	2.4 ± 1.0	1.2 ± 0.3
L-GH	2.61 ± 0.18	1.16 ± 0.18	13.6 ± 3.0	18.4 ± 3.3	699.1 ± 262.6	17.6 ± 3.8	2.8 ± 1.2	1.2 ± 0.3
M-GH	2.35 ± 0.22	1.04 ± 0.28	16.0 ± 3.0	21.3 ± 3.5	884.2 ± 220.4	18.5 ± 4.5	3.0 ± 1.1	1.3 ± 0.3
H-GH	2.74 ± 0.39	1.28 ± 0.34	12.3 ± 2.8	17.9 ± 4.3	675.5 ± 191.0	17.7 ± 4.5	2.2 ± 0.6	1.0 ± 0.1

Values are expressed as mean $\pm$ S.D. ($n=5$)

*Significantly different from control ($p<0.05$).

Control, treated with vehicle; L-GH, treated with 2 IU/kg/time; M-GH, treated with 6 IU/kg/time; H-GH, treated with 20 IU/kg/time.

MAR, mineral apposition rate; Aj. AR, adjusted apposition rate; dLS/BS, double-labeled surface/bone surface; MS/BS, mineralizing surface/bone surface; BFR/TV, bone formation rate/tissue volume; BFR/BS, bone formation rate/bone surface; Mlt, mineralization lag time; Omt, osteoid maturation time.

giosa between 1,760 μm and 2,200 μm distal to the GPMJ (Table 12), the M-GH group showed a significantly higher total TRAP-positive cell number per tissue area, and the H-GH group showed a significantly lower TRAP-positive cell numbers (multinuclear and total) per bone volume, but these were the only significant differences observed between the control and GH-treated groups.

Discussion

Mini rats, a transgenic rat strain produced by introducing a rat GH antisense cDNA into Wistar rat embryos, have been reported to express the rat GH antisense RNA in the pituitary, liver, spleen, and thymus, and to show lower plasma GH levels than Wistar rats^{1,2}. We previously reported that the femurs of Mini rats were smaller and had lower

mineral densities than those of Wistar rats, and that they were histologically characterized by a reduction of the metaphyseal and diaphyseal bone¹². In another study to examine and compare hind limbs (femur and tibia) from these two strains of rats at 10 to 60 weeks of age, we found, first, that bone size, mineral content, and mineral density of the hind limbs were lower in Mini rats, and second, that the amounts of metaphyseal cancellous and diaphyseal cortical bone mass were lower in Mini rats. The results of bone histomorphometry on the proximal and diaphyseal tibia with fluorochrome labeling of the bone suggested that the reductions of bone mass in the metaphysis and diaphysis in Mini rats may be attributable to the lower longitudinal growth rate and periosteal bone formation rate, respectively, and further, that there was no difference in bone turnover in the secondary spongiosa, an area where bone remodeling may occur. It was

Table 9. Static and Dynamic Cortical Bone Histomorphometry of Diaphyseal Tibia in Mini Rats Treated Intraperitoneally with GH

Group	T. Ar (mm ²)	Ps. Pm (mm)	Ct. Ar (mm ²)	Ec. Pm (mm)	Ma. Ar (mm ²)	Ps. MAR (μ m/day)	Ps. MS /BS (%)	Ps. BFR /(mm ³ /cm ² /year)	Ec. MAR (μ m/day)	Ec. MS /BS (%)	Ec. BFR /BS (mm ³ /cm ² year)
Control	3.13 $\pm$ 0.12	8.18 $\pm$ 0.61	2.25 $\pm$ 0.08	3.70 $\pm$ 0.19	0.88 $\pm$ 0.05	1.61 $\pm$ 0.17	47.8 $\pm$ 5.6	28.3 $\pm$ 5.0	1.27 $\pm$ 0.34	26.1 $\pm$ 15.8	13.3 $\pm$ 11.1
L-GH	3.25 $\pm$ 0.14	7.69 $\pm$ 0.67	2.34 $\pm$ 0.14	3.93 $\pm$ 0.25	0.90 $\pm$ 0.12	1.77 $\pm$ 0.13	60.9* $\pm$ 7.9	39.3 $\pm$ 4.7	1.55 $\pm$ 0.36	29.2 $\pm$ 12.8	17.5 $\pm$ 10.3
M-GH	3.16 $\pm$ 0.18	8.50 $\pm$ 0.94	2.29 $\pm$ 0.14	4.04 $\pm$ 0.82	0.87 $\pm$ 0.05	2.08* $\pm$ 0.21	54.2 $\pm$ 6.8	41.3 $\pm$ 8.9	1.79 $\pm$ 0.57	23.7 $\pm$ 3.7	15.8 $\pm$ 6.5
H-GH	3.20 $\pm$ 0.23	7.79 $\pm$ 0.44	2.32 $\pm$ 0.18	3.76 $\pm$ 0.31	0.88 $\pm$ 0.05	2.46* $\pm$ 0.28	75.0* $\pm$ 6.5	67.8* $\pm$ 11.7	1.22 $\pm$ 0.25	15.8 $\pm$ 5.3	7.2 $\pm$ 3.4

Values are expressed as mean $\pm$ S.D. ($n=5$) ND, not determined.

*Significantly different from control ($p<0.05$).

Control, treated with vehicle; L-GH, treated with 2 IU/kg/time; M-GH, treated with 6 IU/kg/time; H-GH, treated with 20 IU/kg/time.

T. Ar, tissue area; Ps. Pm, periosteal perimeter; Ct. Ar, cortical bone area; Ec. Pm, endocortical perimeter; Ma. Ar, marrow area; Ps. MAR, periosteal mineral apposition rate; Ps. MS/BS, periosteal mineralizing surface/bone surface; Ps. BFR/BS, periosteal bone formation rate/bone surface; Ec. MAR, endocortical mineral apposition rate; Ec. MS/BS, endocortical mineralizing surface/bone surface; Ec. BFR/BS, endocortical bone formation rate/bone surface

Table 10. TRAP-Positive Cell Number in Distal Femoral Metaphysis in Mini Rats Treated Intraperitoneally with GH (Growth plate-metaphyseal junction)

Group	TRAP-positive cell number/T. Ar (/mm ²)		
	Mono	Multi	Total
Control	68.8 $\pm$ 9.4	51.8 $\pm$ 9.9	12.6 $\pm$ 13.3
L-GH	71.1 $\pm$ 9.9	57.1 $\pm$ 8.3	128.3 $\pm$ 8.1
M-GH	58.4 $\pm$ 7.9	48.0 $\pm$ 7.1	106.3 $\pm$ 11.9
H-GH	61.2 $\pm$ 10.2	58.4 $\pm$ 18.7	119.5 $\pm$ 18.0

Values are expressed as mean $\pm$ S.D. ($n=5$)

*Significantly different from control ($p<0.05$).

Control, treated with vehicle; L-GH, treated with 2 IU/kg/time; M-GH, treated with 6 IU/kg/time; H-GH, treated with 20 IU/kg/time.

Mono, TRAP-positive mononuclear cells; Multi, TRAP-positive multinuclear cells; Total, TRAP-positive mononuclear cells+TRAP-positive multinuclear cells; T. Ar, tissue area.

briefly suggested that treatment with GH may not affect bone remodeling, as reported before¹³, but other reports have contended that GH does have a stimulatory effect on bone turnover and bone formation⁹⁻¹¹. In the present study, therefore, we administered GH to Mini rats at three dose levels, measured the bone size, mineral content, and mineral density of the bone removed, and performed bone histomorphometry in order to investigate the effects of GH on bone, including bone modeling and remodeling.

Body weight in the GH-treated groups tended to increase in a dose-related manner in the present study. Organs prominently effected by treatment with GH were the

spleen, liver, and adrenals, and their absolute and relative weights were increased. When hypophysectomized rats were treated intraperitoneally with GH at a dose of 2 IU/kg/time twice daily for 14 consecutive days by our group, relative weights of the spleen and thymus were significantly increased²⁰. In the present study, the spleen weight was increased in the GH-treated groups but the thymus weight did not show any significant change. Hematologically, PLT count was increased significantly in the M-GH and H-GH groups, WBC count tended to be increased in the H-GH group, and histologically increased hematopoiesis in the bone marrow was observed in all GH-treated groups. Thus, the increased weights of the spleen and thymus might be related to the increased hematopoiesis in these organs. Hypocellularity was observed in the bone marrow of both hypophysectomized and dwarf rodents, and apparently this hypocellularity could be restored by GH replacement²¹. Thus, we can presume that treatment with GH in Mini rats may bring about increased hematopoiesis with increased cellularity in the bone marrow.

In blood chemical examination, IP tended to be higher in all GH-treated groups, and ALP tended to be higher in the M-GH and H-GH groups. Since GH is known to stimulate reabsorption of IP in the kidney²², high values of IP in GH-treated groups were considered to be an effect of GH. Since the GH-treated groups consumed more or less the same amount of food as the control group (data not shown), the high ALP values might have been related to the increased bone formation in the periosteum and increased endochondral ossification, as described below.

Bone lengths of the femur and tibia tended to be greater in GH-treated groups, but diaphyseal diameters of the femur showed no significant differences. The latter finding may have been related to the shorter period of the treatment with GH. According to bone histomorphometry, periosteal bone formation tended to be higher in GH-treated groups, as described below. Bone mineral content and mineral density were significantly higher in the femurs of the H-GH group overall and at all portions, and in the tibiae of the H-GH

Table 11. TRAP-Positive Cell Number in Distal Femoral Metaphysis in Mini Rats Treated Intraperitoneally with GH (Secondary spongiosa-880 μ m distant from growth plate-metaphyseal junction)

Group	TRAP-positive cell number/B. Pm (/100 mm)			TRAP-positive cell number/B. Ar (/mm ²)			TRAP-positive cell number/T. Ar (/mm ²)		
	Mono	Multi	Total	Mono	Multi	Total	Mono	Multi	Total
Control	355.6 ± 76.2	396.1 ± 116.3	751.7 ± 134.3	125.2 ± 35.6	139.4 ± 50.8	264.6 ± 68.8	43.4 ± 6.7	49.3 ± 17.1	92.8 ± 19.4
L-GH	455.8 ± 127.6	376.5 ± 78.3	832.3 ± 93.6	162.6 ± 64.8	112.4 ± 40.5	275.0 ± 80.5	56.0 ± 13.4	63.5 ± 39.2	119.4 ± 34.9
M-GH	333.8 ± 41.4	340.7 ± 80.9	674.5 ± 67.1	96.9 ± 9.0	99.8 ± 27.7	196.7 ± 23.0	45.9 ± 9.2	46.2 ± 11.5	92.1 ± 13.7
H-GH	339.0 ± 54.2	359.4 ± 82.3	698.4 ± 120.3	107.4 ± 34.2	111.8 ± 31.4	219.2 ± 62.6	49.7 ± 10.8	52.5 ± 12.6	102.2 ± 21.3

Values are expressed as mean $\pm$ S.D. ($n=5$)

*Significantly different from control ($p<0.05$).

Control, treated with vehicle; L-GH, treated with 2 IU/kg/time; M-GH, treated with 6 IU/kg/time; H-GH, treated with 20 IU/kg/time.

Mono, TRAP-positive mononuclear cells; Multi, TRAP-positive multinuclear cells; Total, TRAP-positive mononuclear cells + TRAP-positive multinuclear cells; B. Pm, bone perimeter; B. Ar, bone area; T. Ar, tissue area.

Table 12. TRAP-Positive Cell Number in Distal Femoral Metaphysis in Mini Rats Treated Intraperitoneally with GH (Secondary spongiosa-1,760 μ m distant from growth plate-metaphyseal junction)

Group	TRAP-positive cell number/B. Pm (/100 mm)			TRAP-positive cell number/B. Ar (/mm ²)			TRAP-positive cell number/T. Ar (/mm ²)		
	Mono	Multi	Total	Mono	Multi	Total	Mono	Multi	Total
Control	471.8 ± 112.9	488.7 ± 111.0	960.5 ± 207.5	169.6 ± 41.0	172.7 ± 26.1	342.3 ± 62.8	30.6 ± 5.7	31.3 ± 2.7	61.9 ± 7.2
L-GH	479.5 ± 77.2	414.4 ± 98.9	893.9 ± 137.2	171.4 ± 60.0	142.2 ± 28.0	313.6 ± 79.5	34.8 ± 8.4	30.6 ± 9.7	65.3 ± 16.6
M-GH	488.8 ± 131.6	478.4 ± 124.9	967.3 ± 200.4	162.4 ± 48.5	155.9 ± 24.3	318.3 ± 52.5	45.2 ± 12.6	44.1 ± 11.6	89.3* ± 19.6
H-GH	435.9 ± 83.0	348.5 ± 56.3	784.4 ± 123.1	130.0 ± 37.9	103.5* ± 29.9	233.5* ± 65.2	45.5 ± 7.9	36.5 ± 5.2	82.0 ± 11.1

Values are expressed as mean $\pm$ S.D. ($n=5$)

*Significantly different from control ($p<0.05$).

Control, treated with vehicle; L-GH, treated with 2 IU/kg/time; M-GH, treated with 6 IU/kg/time; H-GH, treated with 20 IU/kg/time.

Mono, TRAP-positive mononuclear cells; Multi, TRAP-positive multinuclear cells; Total, TRAP-positive mononuclear cells + TRAP-positive multinuclear cells; B. Pm, bone perimeter; B. Ar, bone area; T. Ar, tissue area.

group overall and at the proximal portion. There seemed to be greater differences in the distal portion of the femur and the proximal portion of the tibia. Longitudinal bone growth is known to occur in these areas through endochondral ossification, and the treatment with GH may have stimulated this process in the manner as described below.

Histopathologically, the growth plate width and the amount of the metaphyseal cancellous bone mass in the distal femur and proximal tibia tended to increase in GH-treated groups. The proportion of the width of the resting zone in the growth plate was seemingly decreased, whereas the proportions of the proliferative and hypertrophic zones were increased together with increases in the numbers of chondrocytes. These increases in the growth plate width were consistent with the results of bone histomorphometry. Since the longitudinal growth rate of bone was increased in a dose-related manner, the differences in the bone length were attributable to the longitudinal growth rate of bone. It has

been reported that longitudinal bone growth is a result of a strictly regulated endochondral ossification process in the long bones²³, and this process has been shown to be regulated by a number of growth mediators such as GH and insulin-like growth factor I (IGF-I)^{3,4}. Some studies have also demonstrated reduced longitudinal bone growth in hypophysectomized rats^{5,24}.

When we measured the area of the secondary spongiosa in which the proximal point from the GPMJ was set equally, 880 μ m distal to the GPMJ, in all groups, BV/TV and Tb.Sp tended to increase and decrease, respectively, in the GH-treated groups. These findings were consistent with the histopathological findings. A tendency of OV/BV to decrease in the GH-treated groups was also observed, but other static histomorphometric parameters related to bone formation or resorption did not differ clearly between the control and GH-treated groups. On the other hand, when the proximal point from the GPMJ was adjusted to the

respective longitudinal bone growth, there were no significant differences between the control and GH-treated groups in the static histomorphometric parameters of cancellous bone, including those just mentioned above. In the dynamic parameters, no significant differences were observed regardless of whether the measured areas were adjusted to the respective longitudinal growth rates. These results suggest that GH may not affect either bone formation or bone resorption in the secondary spongiosa. In particular, the measured area adjusted to the respective longitudinal growth rate was the area where double fluorescent labels were seen along the bone surface and where lamellar bone formation occurred, indicating that bone remodeling may have occurred. These results, therefore, strongly suggest that GH does not affect bone remodeling.

Using Mini rats and their parental Wistar strain, we performed bone histomorphometry on the secondary spongiosa of the proximal tibia where lamellar bone formation occurred. The results obtained suggested that the difference in the cancellous bone mass of the secondary spongiosa between these two strains may have been attributable to the difference in the longitudinal bone growth (endochondral ossification) in the growth plate, but not to the difference in bone turnover in the secondary spongiosa¹³. Similar results were obtained in hypophysectomized rats^{5,24}, and hypophysectomized rats have also been reported to show a decreased cancellous bone mass, a decreased trabecular number, and an increased trabecular separation compared with intact rats without any apparent differences in bone surface-referent eroded surface, labeled surface, mineral apposition rate, and bone formation rate²⁴.

There were no clear differences in N.Oc/B.Pm and N.Oc/B.Ar in the secondary spongiosa of the proximal tibia between the control and GH-treated groups regardless of whether the measured areas were adjusted to the respective longitudinal growth rates. Nor were there any differences between the control and GH-treated groups in the numbers of TRAP-positive cells at the GPMJ/primary spongiosa or in the numbers of TRAP-positive cells at the secondary spongiosa between 880 μ m and 1,320 μ m distal to the GPMJ. Although the number of total TRAP-positive cells per tissue area in the M-GH group and the numbers of multinuclear and total TRAP-positive cells per bone volume in the H-GH group were significantly higher, the number of TRAP-positive cells per bone perimeter did not differ between the control and GH-treated groups. It has been reported that the TRAP-positive cell number was decreased in the tibial metaphysis of hypophysectomized rats, but that treatment with GH tended to gradually restore this reduction²⁵. The results of the present study suggest that treatment with GH may not affect the osteoclast number in Mini rats. The difference in the effect of GH replacement on osteoclasts between Mini rats and hypophysectomized rats might derive from the difference in the extent of GH deficiency or in changes in hormones other than GH induced by hypophysectomy. In comparison between Mini rats and Wistar rats, the osteoclast number in the metaphyseal cancellous bone did not differ^{12,13}, possibly suggesting that the osteoclast number at the normal basal level may not be influenced by GH treatment.

There were no significant differences between the control and GH-treated groups in the static histomorphometric parameters of tibial diaphyseal cortical bone. On the other hand, when we looked at dynamic histomorphometric

parameters, Ps.MAR, Ps.MS/BS and Ps.BFR/BS were sometimes significantly higher in the GH-treated groups than in the control group, but there were no differences in their endocortical counterparts (Ec.MAR, Ec.MS/BS, Ec.BFR/BS). While there were differences in the dynamic parameters between the control and GH-treated groups, there were no differences in T.Ar and Ct.Ar. This dissociation can be attributed to the short period of the treatment with GH. In a comparison between Mini rats and Wistar rats, there were no differences between endocortical dynamic parameters, but the static parameters T.Ar and Ct.Ar and the periosteal dynamic parameters Ps.MAR and Ps.BFR/BS were all lower in the Mini rat strain. Thus, it was confirmed on the basis of the histomorphometric data that periosteal bone formation plays an important role in increasing cortical bone mass and diaphyseal diameters¹³. It has also been reported that bone formation in the periosteum and endosteum was decreased in hypophysectomized rats, while bone resorption was increased²⁶. On the other hand, it is suggested that GH may stimulate periosteal bone formation in hypophysectomized rats without having influence whatsoever on endocortical bone formation²⁰. In any case, it is certain that periosteal bone formation plays an important role in increasing the cortical bone mass and diaphyseal diameters.

In conclusion, GH-treated groups showed dose-related increases in the metaphyseal cancellous bone mass, growth plate width, longitudinal growth rate, and periosteal bone formation accompanied by increases in bone size, mineral content, and mineral density. In the GH-treated groups, bone histomorphometric parameters related to bone turnover in the secondary spongiosa of the metaphysis, where lamellar bone formation occurred, were not significantly different from those observed in the control group, thereby suggesting that GH may not have any influence on bone remodeling. It is also suggested that GH replacement in Mini rats may not influence the number of osteoclasts involved in bone resorption.

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