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No effect of growth hormone on recovery of load-protected bone

Cortical bone mass and strength studied in rabbits

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The effect of human growth hormone on the recovery of a previously atrophied diaphyseal bone, stress-shielded by plating, was studied in 56 adult rabbits using the contralateral tibia as a control. At removal of the plates, an initial 40 per cent reduction of torsional strength was seen. The strength was normalized in 3 weeks. A concomitant reduction of bone mineral density was normalized in 1.5 weeks. No difference in the rate of strength regeneration was found between growth hormone-treated rabbits and controls. In the treated rabbits an increase in cortical bone area due to subperiosteal new bone formation was seen in previously plated bones, as well as in sham-operated bones.

Rigid internal fixation results in atrophy of diaphyseal bone (Strömberg and Dalén 1976, 1978, Slätis et al. 1978, Terjesen and Benum 1983, Terjesen et al. 1985) by protection of the bone from mechanical loading and deformation resulting in decreased torsional strength (Strömberg and Dalén 1976). Recovery of the bone strength occurs following removal of the plate (Låftman et al. 1980).

Growth hormone is known to cause increased bone turnover and an increase in bone mass (Frost 1962, Harris et al. 1972, Mankin et al. 1978), and has been reported to enhance the healing of fractures exhibiting signs of delayed union (Koskinen et al. 1978, Ahl & Kalén 1979).

We have studied the morphologic and mechanical events during regeneration of diaphyseal bone in the rabbit after rigid internal fixation, notably to investigate whether growth hormone can influence this process.

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Material and methods

Fifty-six healthy adult rabbits (Swedish land breed) of both sexes weighing 3,250–4,750 grams and approximately 1 year's old were used. The rabbits were anesthetized with Mebumal Vet (ACO, Solna, Sweden; 0.3 ml/kg). All the operations were performed under sterile conditions. On both tibiae, metal plates were attached to the medial aspect of the mid-diaphysis with four screws without compression. The stainless steel plates (ISI 316) were designed in accordance with the AO standard and measured 35×5×2 mm. The screws were AO cortical screws (Strauman HC, Waldenburg, Switzerland), 2-mm wide and 10–15-mm long. The plate on one tibia, chosen at random as a control, was removed and the screws reinserted into their holes. The animals were operated on sequentially, one from each group. In every second animal the plate was removed from the right tibia and in every second animal from the left tibia.

Twelve weeks after plate fixation, the plates were removed at a second operation, and the

screws were reinserted into their holes. On the control tibia, a sham operation was performed – the screws were loosened and then retightened.

Thirteen animals died of anesthetic complications at the first or second operation. The remaining animals withstood the treatment well.

Tetracycline (Terramycin 50 mg/kg body weight) was given as a single intramuscular injection after both operations.

Starting on the day of plate removal, half of the rabbits (test group) were given 0.03 IE per kg body weight of human growth hormone, hGH (Crescormone, Kabi Vitrum AB, Stockholm, Sweden) as a daily intramuscular injection. The other half of the animals (control group) received the same volume of physiologic saline solution.

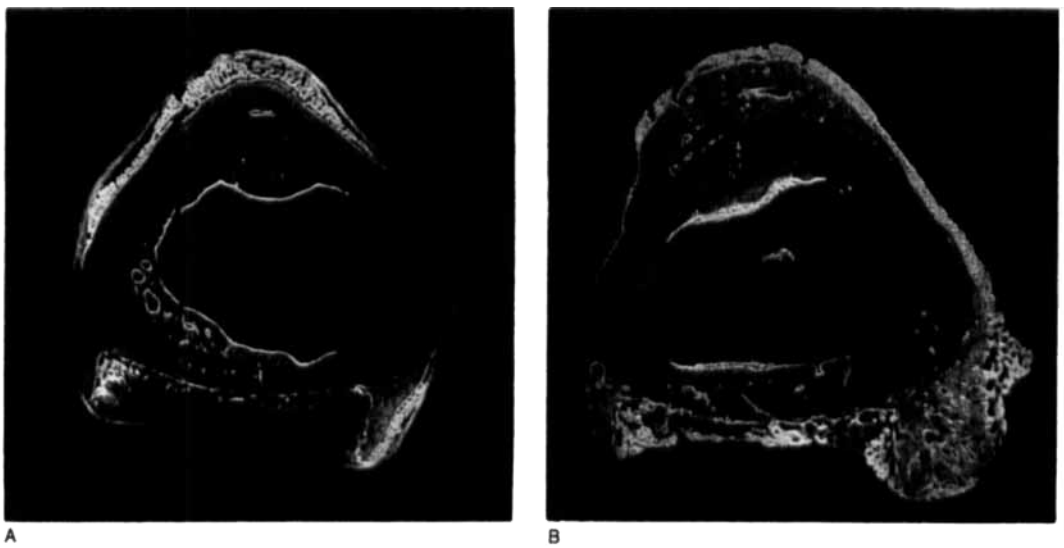
The rabbits were divided into subgroups of 6 or 7 animals. They were killed with a lethal dose of Mebumal Vet. at 1.5, 3, and 6 weeks after plate removal.

Torsional tests. All the tibiae were tested within 30 min after death (Strömberg and Dalén 1976a, b, Jonsson and Strömberg 1980) with the screws in place. Torsional strength was measured as the maximal torque capacity on inward twist at 6°/sec.

Densitometry. The bone mineral content in the transverse segment of the diaphysis previously covered by the plate and the corresponding part of the control bone was measured with the ^{125}I gamma ray absorption technique. Because all the soft tissues were removed from the bones, there was no need for more than one radioactive isotope source. The mid-diaphyses underlying the plate were cut out and embedded in methylmethacrylate. The embedded specimens were scanned from side to side using a multichannel analyzer Norkia LP 4840 densitometer. The Nokia multichannel system was connected to a Honeywell Bull 66/20 computer (Kairento 1979). The values were expressed as grams of mineral per mm^3 of bone.

Planimetry. Eighty-micron-thick cross-sectional slices were cut from the methylmethacrylate-embedded bones with a saw microtome (Leitz). The slices were taken from the bone between the two screw holes closest to the previous center of the plate. These slices were photographed in a fluorescence microscope (Leitz). The photographs were then projected on a graphic tablet connected to an Apple IIe computer and outlined.

The total bone area was calculated as the area contained within the outer periosteal margin of the bone. The medullary cavity was defined as the area inside the endosteal margin of the bone. The cortical bone area was calculated as the difference between the total bone area and the medullary cavity. The newly formed bone could be clearly delineated from the old bone in the fluorescence microscope (Figure 1). The new bone area was



A B

Figure 1. UV-light photomicrograph of cross section of tibia three weeks after removal of the rigid plate. Tetracycline was given at the time of plate fixation, and at removal of the plate. Newly-formed bone is clearly outlined by the fluorescent light.
A. Rabbit treated with saline from the time of plate removal.
B. Rabbit treated with daily injections of human growth hormone from the time of plate removal.

calculated as the bone formed subperiosteally from the second operation until the animals were killed.

Torsional tests were performed on tibiae from 43 animals. Due to technical shortcomings during the preparation procedures, bone specimens from 5 rabbits were excluded from planimetry and 6 from densitometry.

Statistics. The ratio between plated and cortical bones was calculated for each variable in each animal. The two-tailed Wilcoxon rank sum test was then used to compare human growth hormone-treated animals with control animals at each time interval from the removal of the plates. Before the experiments, $P < 0.05$ was designated as being significant.

Results

The animals did not gain weight during the experiment. There were no differences in body weight between the test and control animals.

Torsional strength. At the time of plate removal, the maximal torque capacity of the plated tibiae was reduced by 40 per cent. Three weeks after the removal of the plates, the bones had regained their strength (Table 1). The rate of recovery of maximal torque capacity in the previously plated tibiae was similar in human growth hormone-treated and control animals. The maximal torque capacity of neither plated nor sham-operated tibiae differed in the test groups as compared with the controls at any time. No effects on stiffness were noted upon comparing the surgical procedures or treated and control animals.

Planimetry. No differences were found between previously plated and the corresponding unplated tibiae in the cortical bone area. Further, no enlargement of the medullary cavity was observed on either side. The values of cortical bone area were but slightly higher in the test animals compared with the corresponding controls, both in sham-operated bones and previously plated bones at 1.5, 3, and 6 weeks after plate removal.

Periosteal new bone formation occurred mainly in areas of bone adjacent to the plate or screws (Figure 1). The new bone area was two to five times greater ($P < 0.05$) in the test group in both plated sham-operated bones when compared with the control group at 3 and 6 weeks.

Densitometry. The mineral content of bone previously covered by the rigid plate was reduced by 14 per cent compared with the sham-operated bones at the time of removal of the plates ($P < 0.05$). This reduction had disappeared 10 days after plate removal (Table 1).

There were no differences between plated and sham-operated bones with the exception of a reduction in the bone mineral density of plated bones in the 3-week test group (Table 1).

Table 1. Maximum torque capacity, bone mineral content (BMC) and bone mineral density (BMD) of rabbit tibiae treated with rigid internal fixation (F) or sham-operated (S), at the time of removal of the rigid plate, and at 1.5, 3, and 6 weeks. The rabbits were treated with human growth hormone (hGH) or placebo. Figures in parentheses denote the number of bones tested

		0 weeks		1.5 weeks				3 weeks				6 weeks			
				Placebo		hGH		Placebo		hGH		Placebo		hGH	
		F	S	F	S	F	S	F	S	F	S	F	S	F	S
Torque	mean	2.55	4.15	3.00	3.68	3.60	4.26	4.29	4.18	4.12	4.47	4.20	4.11	4.38	4.39
(Nm)	SD	0.75	0.82	0.56	0.56	0.38	0.45	0.69	0.39	0.61	0.82	0.46	0.21	0.66	0.65
		(7)	(7)	(6)	(6)	(6)	(6)	(5)	(5)	(6)	(6)	(6)	(6)	(7)	(7)
%Difference	mean	38		17		15		−2		6		2		0	
op./sham	SD	17		14		10		22		16		7		10	
BMC	mean	0.56	0.65	0.66	0.67	0.67	0.72	0.74	0.74	0.76	0.78	0.68	0.70	0.70	0.71
(g)	SD	0.05	0.05	0.10	0.04	0.09	0.04	0.04	0.03	0.11	0.07	0.07	0.04	0.06	0.06
		(6)	(6)	(5)	(5)	(6)	(6)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
BMD	mean	2.15	2.44	1.97	2.00	1.73	1.84	1.95	2.13	1.84	2.12	2.05	2.17	1.97	2.06
(g/mm^3)	SD	0.25	0.27	0.19	0.04	0.28	0.15	0.07	0.22	0.03	0.08	0.12	0.43	0.11	0.15
		(6)	(6)	(5)	(5)	(6)	(6)	(5)	(5)	(4)	(4)	(5)	(5)	(5)	(5)

Discussion

The ability of bone to adapt to functional demands is most striking. Little is known about the mechanisms that mediate the translation of load/deformation into a biological response. Growth hormone is known to affect the metabolism of bone; it is necessary for the normal development and growth of bone (Thorngren 1973). It also increases formation and resorption of bone in the adult skeleton resulting in a net increase in bone mass (Frost 1962, Harris et al. 1972, Mankin et al. 1978). However, growth hormone does not seem to to affect the healing of fresh fractures (Harris et al. 1975, Lindholm et al. 1977, Northmore-Ball et al. 1980), although it has been reported to enhance the healing of delayed union (Koskinen et al. 1978, Ahl & Kalén 1979). This caused us to study the effects of growth hormone on the recovery process of atrophied cortical bone subjected to physiologic loading after a period of load protection in rabbits. Human growth hormone was used because it is also known to have an effect in lower mammals (Geschwind 1966, Wittbjer et al. 1984).

The atrophy of diaphyseal bone protected from load and deformation by a rigid internal fixation plate is a result of resorption of cortical bone covered by the plate resulting in widening of the medullary cavity, and decreased strength (Akeson et al. 1976, Paavolainen et al. 1978, Slätis et al. 1978, Strömberg and Dalén 1978, Låftman et al. 1980, Terjesen and Benum 1983, Terjesen et al. 1985). After removal of the plate, the atrophied bone will be subjected to an increased loading, a readaptation process occurs, and strength gradually returns (Låftman et al. 1980).

In the present study an approximate 40 per cent decrease in the torsional strength of plated tibiae occurred. However, no widening of the medullary cavity, as found by others in dogs (Akeson 1976, Strömberg and Dalén 1978), and in rabbits (Paavolainen et al. 1978, Slätis et al. 1978) was found in the plated tibiae at the time of plate removal. This may be due to differences in test animals and to the shorter period of plate protection of the underlying bone in the present study. Further, the recovery of strength in the previously plated tibiae as compared with the sham-operated ones could not be related to changes in cortical or medullary bone area.

The bone mineral content was reduced at the time of plate removal, whereas the cortical bone area remained unaffected, resulting in a decrease in bone mineral density. In a previous study, no change was found in the ratio between the amount of bone mineral and organic material during the process of recovery from atrophy (Låftman and Strömberg 1985). We interprete these findings as signs of osteopenia in the load-protected cortical bone. Regeneration of torsional strength, and of bone mass, occurred within 3 weeks after removal of the plates.

In the present investigation human growth hormone did not affect the rate of return of torsional strength in the previously atrophied tibiae. However, there was an enlargement of the cortical bone area in both plated and sham-operated bone due to increased new bone formation in the groups treated with human growth hormone. Because the new bone formation took place subperiosteally, at the site of the plate or the screws, it was considered to be a result of the surgical trauma. Thus, human growth hormone appears to have an enhancing effect on the periosteal reaction to the surgical procedure. This is in agreement with the findings of Harris et al. (1972) who noted massive periosteal bone formation in dogs treated with growth hormone.

In conclusion, human growth hormone enhanced periosteal new bone formation resulting from mechanical trauma to the periosteum, whereas the rate of recovery of torsional strength of the atrophied diaphyseal bone was not affected.

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