How cancellous and cortical bones adapt to loading and growth hormone

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Abstract

There is great interest in the relationships between growth hormone (GH), muscle loading and bone, in part, because GH increases muscle mass which provides the largest signals that control bone modeling and remodeling. This study was designed to examine the effects of GH and muscle loading by exercise (EX) independently and in combination on bone and skeletal muscle. Thirteen-month-old female F344 rats were divided into 6 groups: Group 1, baseline controls (B); Group 2, age-matched controls (C); Group 3, GH treated (2.5 mg rhGH/kg b. wt/day, 5 days per week); Group 4, voluntary wheel running exercise (EX); Group 5, GH+EX, and rats in Group 6 were food restricted (FR) to lower their body weight and examine the effects of decreased muscle load on bone. All animals, except the baseline controls, were sacrificed after 4.5 months. Growth hormone increased the body weight and tibial muscle mass of the rats markedly, while EX caused a slight decrease in body weight and partially inhibited the increase caused by GH in the GH+EX group. Food restriction greatly decreased body weight below that of age-matched controls but neither FR nor EX had a significant effect on the mass of the muscles around the tibia. Growth hormone and EX independently increased tibial diaphyseal cortical bone area ($p<0.0001$), cortical thickness ($p<0.0001$), cortical bone mineral content ($p<0.0001$), periosteal perimeter ($p<0.0001$) and bone strength-strain index (SSI) ($p<0.0001$). The effects of GH were more marked, and the combination of GH and EX produced additive effects on many of the tibial diaphyseal parameters including bone SSI. GH+EX, but not GH or EX alone caused a significant increase in endocortical perimeter ($p<0.0001$). In the FR rats, cortical bone area and cortical mineral content increased above the baseline level ($p<0.0001$) but were below the levels for age-matched controls ($p<0.0001$). In addition, marrow area, endocortical perimeter and endocortical bone formation rate increased significantly in the FR rats ($p<0.01$, $p<0.0001$, $p<0.0001$). Three-point bending test of right tibial diaphysis resulted in maximum force (Fmax) values that reflected the group differences in indices of tibial diaphyseal bone mass except that GH+EX did not produce additive effect on Fmax. The latter showed good correlation with left tibial diaphyseal SSI ($r=0.857$, $p<0.0001$) and both indices of bone strength correlated well with tibial muscle mass ($r=0.771$, Fmax; $r=0.700$, SSI; $p<0.0001$). We conclude that the bone anabolic effects of GH with or without EX may relate, in part, to increased load on bone from tibial muscles and body weight, which were increased by the hormone. The osteogenic effects of EX with or without GH may relate, in part, to increased frequency of muscle load on bone as EX decreased body weight ($p<0.05$) but had no significant effect on tibial muscle mass. The enhanced loss of endocortical bone by FR may relate, in part, to decreased load on bone due to low body weight ($p<0.0001$) as FR did not cause a significant decrease in tibial muscle mass ($p=0.357$). The roles of humoral and local factors in the bone changes observed remain to be established.

Keywords: Growth Hormone, Exercise, Bone

Introduction

Factors that increase muscle mass (strength) and load on bone cause location specific increase in bone mass and decreased loading of bones results in bone loss[1-3]. Growth

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hormone (GH) and appropriate exercise (EX) can cause muscle hypertrophy[4, 5]; the latter by increasing muscle load on bone may underlie the effects of GH and EX on bone[6]. In reality, the relationships between GH and bone are complex, and the nature of these relationships are not yet fully defined. A clarification of the effects of GH on bone must account for not only its effects on muscle, but also for its effects on humoral IGF-I from liver and local IGF-I in bone, both of which are stimulated by GH which could also

exert a direct effect on bone[7]. In addition, there are uncertainties about the relative responsiveness of cancellous and cortical bone to GH and EX that warrant exploration. The aim of this study was to examine the effects of GH and EX independently and in combination on cancellous and cortical bone and on skeletal muscle in middle aged rats. A group of rats were food restricted to lower their body weight and examine the effects of decreased muscle load on bone as well. In this study we employed voluntary wheel running exercise which is likely to generate unusual patterns of bone strains that are more osteogenic than those produced by flatbed treadmill EX[1]. Three experiments are presented.

Experiment 1

In the first experiment, 13-month-old female Fisher 344 rats were divided into the following 6 groups: Group 1, baseline controls (B); Group 2, age-matched controls (C); Group 3, GH treated (GH); Group 4, voluntary wheel running exercise (EX); Group 5, GH+EX, and Group 6, food restricted (FR). The latter were fed 60% of the mean ad libitum food intake. Group 1 rats were sacrificed at the beginning of the study to serve as baseline controls. Rats in groups 3 and 5 received 2.5 mg rhGH/kg body weight per day, subcutaneously, 5 days per week. The hormone was given in two divided doses of 1.25 mg at 9-10 a.m. and 4-5 p.m. in a volume of 1 ml/kg body weight. Groups 2, 4 and 6 received equivalent volume of the solvent in which GH was dissolved. Groups 2 – 6 were sacrificed after 4.5 months.

Body weight

The six groups of rats studied started with similar initial mean body weight. The treatment regimens produced predictable changes in body weights. At the termination of the study, the heaviest rats were those in the GH group. Exercise caused a decrease in body weight in the GH+EX group relative to the GH group, while the group that was only exercised (EX) maintained a slightly lower body weight than the age matched controls. The FR group lost weight and weighed the least during the experimental period.

Weight of adipose tissue and "tibial muscle"

Following sacrifice, the perirenal adipose mass of each rat was removed and weighed to gain some insight into the effects of the treatment regimens on body fat. The differences in body weight were due, in part, to effects on body fat. Except for GH therapy alone, the various treatments caused a highly significant decrease in perirenal adipose tissue (p<0.0001). The level of the decrease was similar in the FR and EX groups. GH therapy further decreased adipose weight in the GH+EX group (p<0.0001). GH therapy caused the expected increase in the weight of "tibial" muscles (gastrocnemius, soleus, extensor digitorium longus, plantaris) while EX and FR had no significant effect

on muscle weight. The increase in tibial muscle weight due to GH was partially inhibited by EX in the GH+EX group. Although the decrease in muscle weight caused by EX was unanticpated, it appears to be real. It was not likely due to a decrease in muscle fat because EX by itself and FR which markedly decreased perirenal adipose mass had no significant effect on tibial muscle weight. The group differences in the wet weight of the tibial muscles persisted after the muscles were dried, indicating that the increase in muscle mass due to GH was not due mainly to increased muscle retention of water.

Tibial cortical area, cortical thickness, cortical mineral content and cortical density assessed by pQCT

Tibial cortical parameters were analyzed by pQCT densitometry using the XCT Research M system. Over the experimental period of 4.5 months, tibial cortical area increased slightly, and the increase was augmented by most of the treatments. The greatest increase was observed in the GH+EX group followed by the GH and EX groups. In the FR group, cortical area increased above the baseline level (p<0.0001) but was lower than the level for age-matched controls (p<0.001).

Similar observations were made in tibial cortical bone thickness and cortical bone mineral content. However, in the former the level for GH+EX group was not significantly higher than for the group given GH alone, and the levels for FR and baseline groups were not significantly different. GH and GH+EX, but not EX alone caused a slight increase in cortical bone density, while FR decreased cortical bone density below the level for age-matched controls. The absolute changes in cortical bone density were small and varied from 1.5% for the GH+EX group to –0.9% for the FR group.

Tibial periosteal perimeter and endocortical perimeter using pQCT

The changes in tibial periosteal perimeter paralleled and are likely responsible for the changes in tibial cortical bone due to GH and EX. Periosteal perimeter increased slightly during the experimental period. The treatment regimens caused a further increase in periosteal perimeter. The greatest increase was due to GH+EX, followed by GH and EX. The periosteal perimeter of FR rats was similar to that of age-matched controls, but significantly higher than the level for the baseline control group. This indicates that the restricted food intake was not limiting to circumferential bone growth. FR and GH+EX, but not GH and EX alone caused a significant increase in endocortical perimeter.

Histomorphometry of the tibio-fibular junction

To gain further insight into the effects of GH and EX on cortical bone, the tibio-fibular junction was examined by histomorphometry. The data obtained were basically similar to the pQCT data obtained from the tibial diaphysis. At the end of the study, bone tissue area, cortical bone area and %

cortical bone area increased above the level for baseline controls. GH therapy further increased bone tissue area, cortical bone area and % cortical bone area significantly, while EX only significantly increased bone tissue area and cortical bone area. GH therapy and EX increased periosteal double label perimeter and periosteal bone formation rate (BFR) but the increase in BFR due to EX alone was not statistically significant. GH+EX significantly increased bone tissue area, cortical bone area and periosteal double label. Food restriction resulted in endocortical bone loss. In the FR group, % cortical bone area decreased below the level for age-matched controls while marrow area (p<0.01), endocortical double label (p<0.0001) and endocortical BFR (p<0.0001) increased significantly.

Physical activity and spontaneous cage activity

The stimulation of endocortical bone loss due to FR was not because the restriction of food intake caused the animals to be less active. In fact, the rats in the FR and GH+EX groups recorded significantly higher spontaneous cage activity than rats in the other groups. The rats in the EX group ran 84,567 ± 21,046 meters and those in the GH+EX group ran 77,678 ± 12,950 meters over the experimental period.

Bone quality: stress strain index of the left tibial diaphysis, and mechanical strength of the right tibial diaphysis

Growth hormone and EX increased tibial bone stress strain index (SSI). The increase due to GH was greater, and GH+EX increased SSI even more. These findings indicate that GH and EX increased the mechanical competence of cortical bone. In contrast, FR partially prevented the slight increase in SSI that occurred in age-matched controls. Tibial diaphyseal bone strength (Fmax) measured by three point bending gave essentially similar results with the exception that with three point bending, the effect of GH+EX was not significantly higher than that of GH alone.

Vertebral cancellous bone assessed by pQCT

The effects of the treatment regimens on cancellous bone were assessed by examining the vertebra which contains an inner core of cancellous bone that is surrounded by cortical bone. The 4th lumbar vertebra was scanned at three sites using pQCT densitometry: at the midpoint, 1 mm from the midpoint cranially and 1 mm from the midpoint caudally. GH increased total vertebral area and total vertebral mineral content with or without EX. Exercise by itself had a positive but modest bone anabolic effect; GH and EX did not have additive effect in the GH+EX group, and the effect of FR was mostly negative. There were regional differences in the effects of EX. Although EX appeared not to increase cancellous bone at the midpoint of the vertebra, in the slices 1 mm from the midpoint, EX increased cancellous bone mineral content and the increase was significant in the caudal end.

Vertebral cancellous and cortical bone densities assessed by pQCT

There were notable differences in vertebral cancellous and cortical bone densities. The highest vertebral bone density values were observed in cortical bone and the lowest values were observed in cancellous bone. Vertebral cortical bone mineral density was relatively stable among the treatment groups (924-983 mg/mm^3). However, the level is slightly lower than the level we observed for cortical bone density of the tibial diaphysis (1.33-1.36 g/mm^3). In contrast to the high level and relative stability of cortical bone mineral density among treatment groups, vertebral cancellous bone mineral densities were very low and there were marked differences between the different treatment groups (25.63-94.02 mg/mm^3). The low value of vertebral cancellous bone density may relate, in part, to the vertebral bone site and the advanced age of the animals. In the tibial metaphysis of the same animals, the range for cancellous bone density was 461-563 mg/mm^3 which is comparable to the reports of other investigators[8, 9]. These data suggest that pQCT measures basically material density for cortical bone and volumetric rather than material density for cancellous bone as was previously noted[9].

Comparison with the findings of others

The findings from this study support, in part, and extend the observations of others[10, 11]. Yeh et al studied the effects of ovine GH therapy and treadmill EX separately and in combination on the tibial diaphysis of female rats for 9 and 16 weeks[10]. At 16 weeks, which is comparable to the duration of our study, EX but not GH increased the indices of bone mass of the tibial shaft. The positive effect of EX was not seen in a combination therapy of GH+EX. While the reason for the latter finding is unclear, the failure of ovine GH therapy to increase bone mass was attributed to the dose of hormone used which was deemed to be too low[10]. In a subsequent study[11] that used a similar dose of rhGH as in our study, GH had similar effects on femoral mid-diaphyseal area as we observed in the tibial diaphysis, but the treadmill EX used in this study[11] did not increase cortical bone area. The fluorochrome labeling findings from our study and the two studies cited above[10, 11] are consistent with a stimulatory effect of GH and EX on osteoblast recruitment on the periosteal surface of the diaphysis of long bones with no substantive effect on endocortical bone except in the GH+EX group.

Experiment 2

Cancellous bone, ovariectomy and GH therapy

In experiment 2 we examined the effects of GH on cancellous bone in ovariectomized rats to determine if it can rebuild bone following loss due to ovarian hormone deficiency as occurs in postmenopausal women. Analysis of the 3rd lumbar vertebra by histomorphometry indicated that

ovariectomy caused the expected decrease in vertebral cancellous bone volume. But unlike our previous finding in ovarian hormone replete rats, GH was ineffective in increasing vertebral cancellous bone in the ovarian hormone deficient animals. These findings indicate that the bone anabolic action of GH is more effective in the presence of sex steroid hormones. However, the apparent lack of response of cancellous bone to GH was not observed in the neck of the femur. These complexities in the actions of GH may underlie some of the difficulties in demonstrating its efficacy in the treatment of osteoporosis.

Cancellous bone, ovariectomy and GH therapy: pQCT vs. histomorphometry

The above data on the relative lack of effect of GH on cancellous bone in ovariectomized rats was generated from histomorphometric analysis of bone sections from the vertebral body. The relatively new technique of pQCT not only complements histomorphometry, it can bring new insight into otherwise puzzling data. In the above study, histomorphometric analysis indicated that GH did not increase cancellous bone in the lumbar vertebra of ovariectomized animals. However, analysis of the vertebra by pQCT densitometry showed an increase in cancellous bone area with no significant increase in cancellous bone mineral content. These findings indicate that pQCT is more sensitive than histomorphometry in determining small changes in cancellous bone, and that the cancellous bone formed by GH therapy in ovariectomized rats appear not to be fully mineralized at the time the experiment was terminated.

Experiment 3

In the final study we inquired whether GH could mediate its skeletal effects, in part, by acting directly on bone. To this end, we examined the immediate effects of GH administration on the expression of the mRNAs of bone matrix proteins and related proteins in bone[7]. Three-month-old female Sprague-Dawley rats were each given a single injection of GH (8 mg/kg b. wt.) and sacrificed 15 min, 1 h, 2 h, 4 h, 8 h, 16 h and 24 h later. Control animals were given solvent vehicle and sacrificed immediately. RNA was isolated from cancellous bone harvested from the distal metaphysis of the femur of all animals. GH increased the level of bone type I collagen mRNA by 187%, 417% and 509% over the control level at 15 min, 1 h and 2 h, respectively; the mRNA levels declined to 119% and 99% at 4 h and 8 h, respectively, and then rose again to 351% and 423% over the control level at 16 h and 24 h, respectively. The level of bone IGF-I mRNA increased by 45%, 83%, 120%, 140% and 175% over the control level at 2 h, 4 h, 8 h, 16 h and 24 h, respectively, following GH administration. In a second experiment, animals received a similar dose of GH as in the preceding experiment and were sacrificed at 0 h, 30 min, 1 h, 2 h and 4 h following injection, and RNA isolated from bone as previously described.

Following GH administration: bone osteocalcin mRNA increased by 127%, 177%, 361% and 413% over the control levels at 30 min, 1 h, 2 h and 4 h, respectively; bone IGF-I mRNAs increased by 38%, 33%, 87% and 437% at 30 min, 1 h, 2 h and 4 h, respectively, but the levels did not become significant until 2 h; bone c-fos mRNA increased significantly at 30 min and returned to baseline at 2 h, while bone c-jun and c-myc mRNAs did not increase until 4 h following GH administration. Serum IGF-I did not increase significantly until 8 h after GH administration.

We conclude that GH stimulates a rapid increase in the expression of mRNAs for the bone matrix proteins, type I collagen and osteocalcin. Since the effects of GH on the mRNAs of these bone matrix proteins preceded its effects on IGF-I and the mRNAs of the early response oncogenes that have been implicated in the mediation of its action, part of the osteogenic effect of GH is mostly likely mediate directly.

Summary and Conclusions

The following summarizes our conclusions from the studies discussed.

Voluntary wheel running exercise

Wheel running is a moderate intensity exercise with osteogenic property. It has the advantage that it is voluntary and free of the stress associated with forced running on flatbed treadmill. It is capable of producing unusual patterns of bone strains that may be more osteogenic than strains produced by flatbed treadmill EX. Voluntary wheel running is, therefore, a potentially useful model for studying the effects of mechanical usage on bone. However, because its osteogenic effect is modest, there is a great need for an effective bone anabolic EX regimen for rodents.

Growth hormone and exercise

GH and EX increased bone mass mainly by increasing osteogenesis on the periosteal envelope. GH is more potent than wheel running EX and acts mainly on cortical bone, but it also affects cancellous bone. The sensitivity of cancellous bone to the anabolic effect of GH varies among bone sites, and appears to be decreased by ovarian hormone deficiency. These complexities may contribute to the disappointing results of investigations of the therapeutic potential of GH in osteoporosis.

Growth hormone and exercise

The bone anabolic effects of GH may relate, in part, to increased load on bone due to increased muscle mass. In addition, GH appears to have a direct bone anabolic action that is independent of IGF-I and the early response oncogenes. The bone anabolic effect of exercise by itself may relate, in part, to increased frequency of muscle load on

bone since EX decreased body weight but did not increase muscle mass.

Food restriction and bone

The enhanced loss of endocortical bone caused by FR may relate to decreased load on bone due to low body weight since FR did not cause a significant decrease in muscle mass. The disuse-like remodeling induced by FR may be a physiological response to reduce bone mass to a level appropriate to the reduced body weight caused by the reduction in food intake.

References

1. Lanyon LE. Using functional loading to influence bone mass and architecture: Objectives, mechanisms, and relationship with estrogen of the mechanically adaptive process in bone. Bone 1996; 18: 37S-43S.
2. Gutin B Kasper MJ. Can vigorous exercise play a role in osteoporosis prevention? A review. Osteoporosis Int 1992; 2: 55-69.
3. Kannus P, Sievanen H, Vuori I. Physical loading, exercise, and bone. Bone 1996; 18 (Suppl): 1-3.
4. Cartee GD, Bohn EE, Gibson, BT Farrar RP. Growth hormone suplementation increases skeletal mass of old male Fischer 344/Brown Norway rats. J Gerontol 1996; 51: B214-219.
5. Willis PE, Chandan SG, Baracos V, Parkhouse WS Restoration of insulin-like growth factor-I action in skeletal muscle of old mice. Am J Physiol 1998; 275: E525-E530.
6. Frost HM. Could some biomechanical effects of growth hormone help to explain its effects on bone formation and resorption? Bone 1998; 23: 395-398.
7. Salih MA, Orhii PB, Chen C, Kalu DN. Growth hormone and the expression of mRNAs for matrix proteins and oncogenes in bone. Molecular and Cellular Endocrinology 1999; 147: 149-159.
8. Breen SA, Millest AJ, Loveday BE, Johnstone D, Waterton JC. Regional analysis of bone mineral density in the distal femur and proximal tibia using peripheral quantitative computed tomography in the rat in vivo. Calcif Tissue Int 1996; 58: 449-453.
9. Gasser JA. Assessing bone quantity by pQCT. Bone 1995; 17(Suppl): 145S-154S.
10. Yeh JK, Aloia JF, Chen M. Growth hormone administration potentiates the effect of treadmill exercise on long bone formation but not on the vertebrae in middle aged rats. Calcif Tissue Int 1994; 54:38-43.
11. Oxlund H, Andersen NB, Ortoft G, Orskov H, Andreassen TT. Growth hormone and mild exercise in combination markedly enhance cortical bone formation and strength in old rats. Endocrinology 1998; 139: 1899-1904.