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Effects of Low-Dose Recombinant Human Growth Hormone on Bone Densities of Radius, Tibia and 4th Lumbar Vertebrae in Rabbits

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Abstract

Objective- To determine the effects of growth hormone (GH) therapy on bone density in controlled conditions in healthy adult rabbits.
Design- Experimental in vivo study.
Animal- 20 healthy, adult New Zealand White rabbits.
Procedures- The rabbits were divided into 2 groups; receiving Human GH (0.006 mg/kg/d) and controls; receiving placebo for 3 months. The density of radius and tibia was measured in the proximal epiphysis, mid shaft and distal epiphysis while the density of vertebral column was measured in the fourth lumbar vertebra (L4) using aluminum step-wedge and appropriate software. Measurements were performed in 5 stages, one before the start of therapy and 4 times after the administration of GH or placebo, with 3 weeks interval.
Results- The mean level of serum insulin-like growth factor I (IGF-I) was increased significantly after GH therapy ($p<0.05$) in the test group (222 ± 51 vs. 270 ± 64 ng/l). The mean level of IGF-I did not change significantly in control group. Densitometric measures in mid shaft of radius was increased significantly in test group after GH therapy while it did not have any significant variation in control group ($p<0.05$).
Conclusion and Clinical Relevance- The difference between the long bones and vertebrae in terms of GH responsiveness is similar to what was reported in human acromegaly. Decrease in density in the second stage in some regions that followed by increasing in the next stages was similar to what was reported in treatment of patients with growth hormone deficiency (GHD). This study suggests that rabbits might be a useful model to assay GH effects on bone density in acromegaly, GHD, or healthy human adult.
Keywords- Growth Hormone, Bone Density, Rabbit.

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Introduction

Effects of GH on bone density in human have shown controversial results.[1-4] This controversy may be in part due to this fact that studies in human could not be done in fully controlled conditions, and many variables could interfere with the results. For example, smoking decreases IGF-I while physical activity increases GH secretion.[5]
GH plays an important part in the maintenance of bone mass in adults.[6-8] GH appears to mediate osteoblastic function in part by the influence of IGF-I.[9,10] Decreased bone mineral density (BMD) is reported in adults with GH deficiency, in whom GH replacement results in increases in bone turnover and BMD.[9,11,12] In acromegaly, bone formation and bone resorption are increased, however different results have been presented regarding the specific effect and site of action in bones in acromegaly.[13-15] Effect of GHD on bone remodeling in adults is inconclusive. Serum levels of osteocalcin, reflecting osteoblast activity and bone formation, have been shown to be decreased[16,17], increased[18] or unchanged[19] in GHD patients.
Evaluating the effect of GH on bone in a standard animal model and in controlled condition could have more reliable results. The purpose of this study was to determine the effects of low-dose GH therapy on bone density in controlled conditions in healthy adult rabbits.

Materials and Methods

This study was approved by the Clinical Research Ethics Committee of Tehran University, Tehran, Iran. We performed a randomized clinical trial on 20 healthy, skeletally mature New Zealand White rabbits with equal number of both sexes, weighing 3.2-3.8 kg. All animals were housed in separate cages under controlled environmental conditions and 12:12h light:dark cycle. They were fed a standard rabbit diet and water *ad libitum.* Rabbits were randomized into two groups of 10 cases comprising five males and five females. Animals in the test group were treated with daily administration of recombinant human GH (Norditropin; Novo Nordisk A/S, Bagsvard, Denmark) 0.006 mg/kg body weight. It was injected subcutaneously three times per week in the evening for 12 weeks. The control animals received injections of 0.1 ml sterile saline solution as placebo. On the day of injection, as well as 2 weeks later, a sample of fasting morning venous blood for plasma IGF-I assay was obtained. The samples were taken 24 h after the last treatment dose of GH. The blood was allowed to clot at 4 °C and was centrifuged, and the serum was stored at –70 °C until the time of analysis. Serum IGF-I was quantified by immunoradiometric assay using IRMA IGF-I kit (Immunotech, Marseille, France). BMD measurements were performed just before and 3, 6, 9 and 12 weeks after starting GH treatment. At each period bone density at radius, tibia and L4 were measured. In the long bones, density at proximal epiphysis, distal epiphysis and mid shaft of the right limbs were measured. For densitometry, lateral radiographs of radius, tibia and L4 were obtained using an X-ray apparatus and Kodak high resolution mammography films. An aluminum step-wedge consisting of 15×0.25mm steps was placed in a fixed position on radiographic cassette at the same level for each exposure and a single pulse snapshot was digitized by computer (Fig 1). The final image was assessed and calibrated by using ***Image-J***[1] software. Mean pixel intensities were measured for each step by placing a square region of interest repeatedly over the image of the step-wedge. To avoid slight changes caused from non specific pixels, the square was place on a blank screen (no bone or step wedge) and regarded as 0 mm-Al. A non linear calibration curve (3rd degree polynomial) was

[1] **ImageJ** is a public domain, Java-based image processing program developed at the National Institutes of Health

produced. All subsequent measurements made on each calibrated image were based on millimeters of aluminum equivalent (mm Al. equi). A square region of interest was placed on proximal epiphysis, mid shaft and distal epiphysis in long bones and on L4 in vertebral column and mean radiographic density was measured for each region and expressed as mm Al equi. Repeatability of this method was previously reported[20], and proven to be excellent. Aluminum wedge reported to has absorption and scatter properties that is similar to bone[21] and used for densitometry in similar research.[22,23]

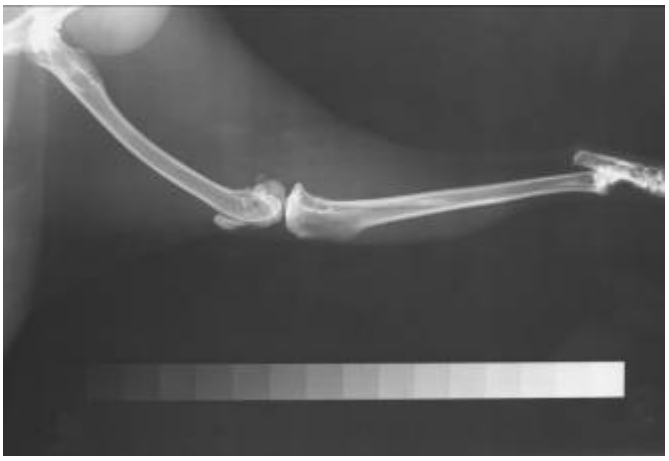

Figure 1. Radiograph taken from right hindlimb of a rabbit to evaluate tibial density using aluminum step-wedge.

Statistical Analysis

Statistical analysis was performed using the SPSS v.16 software (SPSS Inc., Chicago, IL USA). Data were reported as mean and standard deviations of mean values with significance level set at $P < 0.05$. After evaluating the homogeneity of variance and normality of data, we used Repeated Measures ANOVA for comparison between means of dependent variable in two groups during five stages of study. Data are presented as Mean ± SD.

Results

There was no significant change in weight between two groups. The mean level of serum IGF-I was 222 ± 51 ng/l before GH administration which increased to 270 ± 64 ng/l following GH administration in the test group. This value slightly decreased in the control group; 273 ± 83 ng/l versus 272 ± 102 ng/l. The densitometric results of the test and control groups in proximal epiphysis, mid shaft and distal epiphysis of the tibia and radius, as well as in L4 were measured (Fig 2). The densitometric results between the test and control groups showed significant differences in mid shaft of radius ($p<0.05$) (Fig 2B). We did not find any interaction between sex and groups in the analysis.

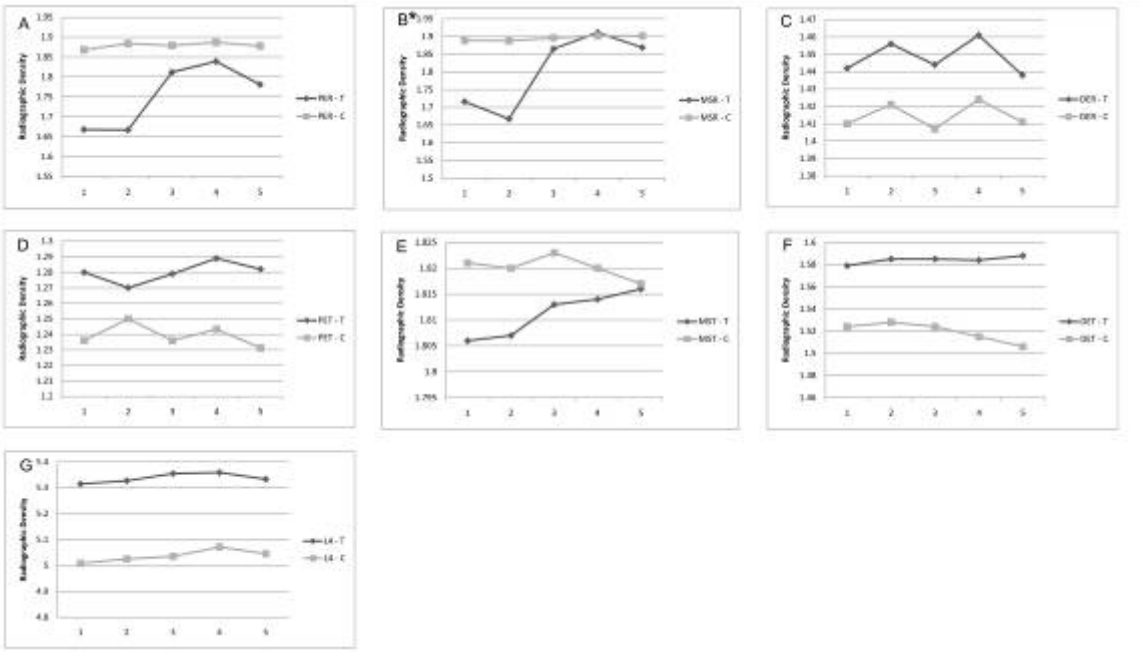

Figure 2. Time-dependent changes in millimeter Aluminum equivalent (mm Al equi) density during GH treatment in (A) proximal epiphysis of radius (PER), (B) mid shaft of radius (MSR), (C) distal epiphysis of radius (DER), (D) proximal epiphysis of tibia (PET), (E) mid shaft of tibia (MST), (F) distal epiphysis of tibia (DET) and (G) L4 in the test (T) and control (C) groups. Significant difference ($p<0.05$) is showed with *.

Discussion

The current study reports effects of 12 weeks low-dose GH injection on bone density in healthy adult rabbit. Serum IGF-I levels increased significantly in test group that showed human GH was effective in rabbits. The densitometric measurements showed significant increase in bone density in mid shaft of radius (Fig 2B) in comparison to control group ($P<0.05$) but changes in vertebrae were not significant. The densities in proximal epiphysis of radius and mid shaft of tibia were increased to some extend but were not significant (Fig 2A, E). Rudman et al.[4] studied the effects of GH replacement therapy in 12 old men for 6 months that improved body composition and Bone Mineral Density (BMD). The density of lumbar vertebrae increased by 1.6% that was statistically significant ($P<0.05$). They could not detect a significant difference in the bone density of the radius or proximal femur. This report encouraged the use of GH as an antiaging intervention.[24] However, later studies showed controversial results.[1] Papadakis et al.[3] reported 0.9% increase in bone mineral content in the GH treated group compared with placebo group that was measured just in lumbar region of healthy old men. Ghiron et al.[2] and Holloway et al.[25] reported increase in markers of bone turnover after administration of GH in healthy elderly women. One study, using old female monkeys, demonstrated that GH, given for 7 weeks, increased bone formation.[26] The effect was seen both in the tibia and in the femur whereas no significant effect was seen in the vertebrae.[26] The difference between the long bones (predominantly cortical bone) and the vertebrae (predominantly cancellous bone) in terms of GH responsiveness is similar to what has been described earlier in old rats.[27]

Rats have been used widely for studying the influence of GH on intact bone, but in almost all of these experiments, GH administration has induced linear bone growth as the growth plates

are open until the rats are very old.[28] Therefore the data have to be evaluated in relation to both growth/modeling and remodeling.[29,30] In contrast, in primates the growth plates are closed after sexual maturation.[27] In rats, GH has been demonstrated to increase periosteal bone formation, vertebral body and femoral diaphysis strength. However, no evidence has been found showing improvements in BMD and Bone Mineral Content.31-33 The rabbit (*Oryctolagus cuniculus*) is a standard laboratory animal and is phylogenetically closer to primates than are rodents.[34] The researches evaluated the effect of GH in rabbit have shown some similarities in the results between rabbit and human.35-38 Many species have a specificity for GH, but most mammals, as well as the rabbit, are sensitive to human GH.[39-40] Based on these evidences rabbit may be a more reliable animal model for evaluating the effects of GH on bone.

In acromegaly there are supraphysiologic levels of GH. There are many studies that have investigated effects of GH on bone in acromegalic patients but the results are controversial and decrease[13] or increase[15] in the density were reported. But generally, most of studies suggesting cortical bone density is increased in acromegaly while trabecular bone seems to be mostly unaffected.[14,41-43] These suggestions are compatible with the results of our study as effects of GH just could be seen in long bones. In addition in the long bones significant effects were seen in mid shaft which is mostly cortical bone.[27] There is one report suggested transgenic rabbits overexpressing GH, are valuable animal models to assay new therapies for acromegaly. Those rabbits showed metabolic, histological, and anatomical alterations that were very similar to those seen in human patients with acromegaly.[36] There is another report suggested transgenic rabbits will be valuable animal models of aberrant bone formation.[35]

Decreased BMD is seen in adults with GH deficiency, in whom GH replacement treatment results in increase bone turnover and BMD.[9,11,12] One study in GHD adults reported that GH treatment at first causing increased bone resorption, results in an apparent low or unchanged bone mass. However, GH treatment after 18 months gives increased bone formation and bone mineralization. Thus, it was concluded that the action of GH on bone metabolism in GHD adults is 2-fold: it stimulates bone resorption in the beginning and bone formation thereafter. Therefore, a biphasic model of GH action in bone remodeling was proposed.[27] In another study on GHD patients treated with growth hormone for seven years, GH shows a triphasic action on BMD: an initial decrease in BMD during the 1st year, followed by a continuous increase in BMD with buildup of a stable plateau after 60 months.[44] Interestingly, although the rabbits of our study were healthy, in the second stage of densitometry after three weeks of treatment, we could identify decrease in bone density in proximal epiphysis of radius and tibia (Fig 2A and D) and in mid shaft of radius (Fig 2B). Bone density increased in all of these regions progressively after six weeks of treatment in the next stages. It is possible that GH did have some effects at the cellular or biochemical level but the effects were insufficient to be manifested radiographically in short term, this could be one reason that we could not detect significant changes in other regions. However, further studies with longer GH treatment and lower or higher dosage are mandatory for a better understanding.

This study reveals low-dose of GH can slightly increase bone density in rabbit and suggests rabbit could be a useful model to assay GH effects on bone in acromegaly, GHD, or healthy adult human.

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چکیده:

اثرات دز پایین هورمون رشد سنتتیک انسانی بر دانسیته استخوان های رادیوس، تیبیا و مهره چهارم کمری در خرگوش

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هدف- ارزیابی اثرات استفاده از هورمون رشد بر دانسیته استخوان در شرایط کنترل‌شده در خرگوش سالم بالغ.
طرح مطالعه- مطالعه تجربی در حیوان زنده
حیوانات- ۲۰ سر خرگوش سفید نیوزیلندی سالم بالغ
روش بررسی- خرگوش‌ها به دو گروه آزمایش و کنترل تقسیم شدند. گروه آزمایش هورمون رشد و گروه کنترل نرمال سالین برای مدت ۳ ماه دریافت کردند. دانسیته استخوان‌های رادیوس و تیبیا در اپی فیز بالایی، وسط شفت و اپی فیز پایینی و دانسیته ستون مهره در مهره چهارم کمری (L4) به وسیله گوه پله‌ای آلومینیمی و نرم افزار مناسب اندازه‌گیری شد. اندازه-گیری‌ها در ۵ مرحله انجام شد، مرحله اول قبل از شروع تجویز هورمون رشد و ۴ مرحله بعد از تجویز هورمون رشد با فواصل ۳ هفته.
نتایج- متوسط غلظت سرمی فاکتور رشد شبه انسولین تیپ یک (IGF-I) در گروه آزمایش بعد از تجویز هورمون رشد به طور معنی داری افزایش یافت ($p<0.05$). دانسیته استخوانی در ناحیه میانی شفت استخوان رادیوس در گروه آزمایش به طور معنی داری افزایش یافت در حالی که در گروه کنترل تغییر معنی داری مشاهده نشد ($p<0.05$).
نتیجه‌گیری و کاربرد بالینی- تفاوت در افزایش دانسیته استخوانی در پاسخ به هورمون رشد که بین ستون مهره و استخوان-های بلند مشاهده شد، با تغییرات دانسیته استخوانی که در آکرومگالی انسان دیده می شود شباهت دارد. همچنین کاهش در دانسیته که در مرحله دوم دیده شد و با افزایش دانسیته در مراحل بعدی دنبال شد با مشاهداتی که در درمان کمبود هورمون رشد در انسان گزارش شده همخوانی دارد. یافته‌های این مطالعه نشان می‌دهد که خرگوش می‌تواند مدل مناسبی برای بررسی اثرات هورمون رشد بر دانسیته استخوان در آکرومگالی، کمبود هورمون رشد و انسان سالم بالغ باشد.
واژه‌های کلیدی: هورمون رشد، دانسیته استخوانی، بالغ، خرگوش.