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Correlation of insulin-like growth factor 1 and osteoarthritic cartilage degradation: a spontaneous osteoarthritis in guinea-pig

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Abstract

OBJECTIVE: The pathogenesis of osteoarthritis centers on the imbalance between catabolic and anabolic processes in cartilage metabolism. Insulin growth factor 1 (IGF-1) has been shown to have anabolic effects in cartilage *in vitro*. This study aim to determine whether IGF-1 on cartilage is associated with loss of chondrocyte and extracellular matrix breakdown using the Hartley guinea pig model.

MATERIALS AND METHODS: Cartilage from the medial and lateral tibial plateau of 6-month and 12-month old Hartley guinea pigs were used for this study. Histological analysis was performed with hematoxylin-eosin (HE) and toluidine blue staining. Safranin-O staining was used to quantify proteoglycan (PG) loss and the extent of cartilage damage by Modified Mankin score. Distribution of IGF-1 was demonstrated with *in situ* hybridization techniques. IGF-1 mRNA levels were assessed using Real-time PCR.

RESULTS: Histological loss of chondrocytes, and cartilage matrix and decreased IGF-1 distribution were demonstrated in a temporal and spatial manner. Compared to the 6-month old samples, the 12-month specimens had significantly cartilage degeneration and less cartilage matrix and PGs staining. Decreased level of IGF-1 was also observed in the 12-month samples. These observations were more pronounced in the medial tibial plateau when compared to the lateral plateau.

CONCLUSIONS: The decreased level of IGF-1 may play a critical role for maintaining the balance between catabolic and anabolic processes in cartilage metabolism during the development of osteoarthritis. Thus, the increase of IGF-1 may be applicable to developing OA therapy.

Keywords

Osteoarthritis; Cartilage; IGF-1; Guinea pig

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Conflict of interest

The authors declare no conflicts of interest.

Introduction

Osteoarthritis is a common affliction that debilitates millions of individuals per year. It is a disease characterized by loss of articular cartilage, hypertrophic bony changes, and inflammatory synovitis. Degenerative changes to articular cartilage are secondary to an imbalance of cartilage homeostasis in which catabolic processes outpace the reparative abilities of cartilage. Although the exact pathogenesis of osteoarthritis has not yet been elucidated, research has shown that mechanical and biochemical forces are responsible for the progression of the disease.

Insulin-like growth factor 1 (IGF-1) is a 70 amino-acid single polypeptide that shares approximately 50% homology with insulin[1]. It is primarily produced by the liver, although most somatic cells in the body can produce the growth factor[2]. Chondrocytes also release IGF-1 and can increase its production during osteoarthritic conditions[3,4]. The IGF-1 receptor is a specific tyrosine kinase receptor that mediates its effects via intracellular signaling. The circulating free level of IGF-1 is determined by several factors. Growth hormone secreted from the anterior pituitary induces IGF-1 production. Calcitropic hormones such as $1,25(OH)_2$ Vit D_3 have also been shown to increase IGF-1 secretion. In osteoarthritic chondrocytes, transforming growth factor B, stimulate IGF-1 secretion[5]. However, the most important factor that affects IGF-1 level is the IGF binding protein (IGFBP), of which IGFBP-3 is the most important. IGF-1 binds to IGFBP-3 to form the labile acid subunit, which acts as the circulatory store of IGF-1[6]. IGFBP-3 has been localized to pericellular matrix in articular cartilage and its level is up-regulated in osteoarthritic cartilage[3,7].

The role of IGF-1 in the body is diverse. It plays critical roles in prenatal development and somatic growth; Laron dwarfism is a condition defined by chronic GH and IGF-1 deficiency that can be treated by supplementing the patient with GH/IGF-1[8]. IGF-1 also augments both innate and acquired immunity by regulating proliferation of lymphocytes, increased TNF-a production, and enhanced NK-cell activity[8]. IGF-1 exerts several effects on cartilage. It induces the production of cartilage matrix components, of which PG and type II collagen are most prominent[9,10].

Scholars have focused on the possible roles of cytokines and growth factors in the development of osteoarthritis. IL-1, TNF-a, and SDF-1 have all been implicated in osteoarthritic development[11,12]. Additional work has focused on the anabolic role of IGF-1 in cartilage metabolism. Despite the *in vitro* evidence supporting the protective role of IGF-1, relatively few studies have been performed in *in vivo* models. Furthermore, studies that attempted to correlate serum IGF-1 levels and severity of osteoarthritis have produced conflicting results[13–15]. Other research has demonstrated an increase of IGF-1 within the synovial fluid of osteoarthritic joints, suggesting that chondrocytes can increase its synthesis of the growth factor during periods of accelerated damage to prevent further progression of the disease[16,17].

The goal of our work is to demonstrate the relationship between the level of IGF-1 and cartilage degradation *in vivo*. We used the Hartley guinea pig, a well-established animal

model for osteoarthritis, to demonstrate that the presence of IGF-1 decreases the extent of chondrocyte and PGs loss and matrix breakdown.

Materials and Methods

Preparation of 6- and 12-Month Samples

6- and 12-month-old outbred male Hartley guinea pigs weighing 0.93±0.05 kg and 1.25±0.06 kg respectively, were used in the experiment (Charles River, Wilmington, MA, USA) N=8 in each group. The animals were killed with intraperitoneal injections of pentobarbital at 6 and 12 months age. The knee joint was harvested and the medial and lateral tibia plateaus were dissected from its soft tissue attachments. The study was approved by the Rhode Island Hospital Animal Welfare Committee (IACUC) (Approval: 0013–08).

Histological Analysis of 6- and 12-Month Samples

The knee joints from 6- and 12- month old animals were fixed overnight in 4% paraformaldehyde in phosphate-buffered saline (pH 7.4), decalcified in 10% EDTA-PBS for 1–2 weeks. Those materials were routinely processed and embedded in paraffin. Serial 5 μm sections were prepared and collected on positively charged glass slides (Superfrost Plus, Thermo Fisher Scientific, Waltham, MA, USA). The sections were dried on a hot plate to increase adherence and then stained with HE to evaluate the histological changes. The samples were stained with toluidine blue to assess proteoglycan content. Safranin-O staining was used to verify PG loss and the cartilage damage. The severity of cartilage damage of each joint was then assessed using the modified Mankin grading system[18]. Three independent observers scored each section and the scores for the medial and lateral tibial condyles were averaged within each joint. All scoring was done in a random order and in a blinded fashion.

The dissector method of Eggli et al[19] was applied to estimate the number of chondrocytes per unit of volume tissue. Briefly, the hematoxylin and eosin stained sample was arithmetically defined into four zones. Two parallel tissue planes separated by a known distance were used. This tissue volume is called a dissector. The number of cells present in section 1 but no longer apparent in section plane 2, divided by the dissector, provided an estimate of number of cells per unit volume.

In Situ Hybridization of IGF-1

A cDNA probe encoding guinea pig IGF-1 was cloned by RT-PCR from total RNA isolated from guinea pig tibia articular cartilage using published IGF-1 mRNA primers 3’-TTCTACCTGGCCTTCTGC-5’ and 3’-GCAGTACATCTCCAGCCT-5’[20]. 6-month-old and 12-month-old male Hartley guinea pigs were used in this study. Eight animals from each age group were used for *in situ* hybridization of IGF-1 mRNA using digoxigenin UTP-labeled single-stranded RNA probes prepared with a DIG-RNA labe ling kit (Cat. No. 11 175 025 910, Roche Applied Science, Indianapolis, IN, USA). Distribution of IGF-I mRNA was detected on paraffin embedded sections.

Real-time PCR of IGF-1

Total RNA was isolated from the tibial cartilages of 6- and 12-month old animals with the RNeasy mini kit (QIAGEN, Valencia, CA, USA) as previously described by Wei et al[21]. 1 μg total of RNA was used for each reverse transcriptase with the iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). Real-time quantitative PCR amplification was performed using QuantiTect SYBR Green PCR kit (QIAGEN, Valencia, CA, USA) with the DNA Engine Opticon 2 Continuous Fluorescence Detection System (MJ Research, Waltham, MA, USA). Primers used in amplification of target genes' mRNA are: 3'-TTCTACCTGGCCTTCTGC-5' and 3'-GCAGTACATCTCCAGCCT-5'. IGF-1 mRNA level was normalized to housekeeping gene 18S RNA levels. Since the level of 18S RNA is constant in all the cells, the normalized values reflected the relative level of the target genes' mRNA in each cell regardless of the cell number. The 18S RNA was amplified at the same time and used as an internal control. The cycle threshold (Ct) values for 18S RNA and that of samples were measured and calculated by computer software (MJ Research, Waltham, MA, USA). Relative transcription levels were calculated as $x = -2\Delta\Delta Ct$, in which $Ct = E - C$, and $E = Ct_{exp}-Ct_{18s}$; $C = Ct_{ctl}-Ct_{18s.}$

Statistical Analysis

SPSS 19.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. All quantitative data were expressed as mean ± standard deviation. Student *t*-test was used to analyze the comparisons between 6- and 12-months groups. p values < 0.05 were considered statistically significant

Results

Chondrocyte Number and Matrix Integrity

Histological analysis of paraffin-embedded samples from the tibial plateau demonstrated decreased cellularity and matrix integrity in a temporal and spatial-dependent manner. The extent of cartilage disruption and proteoglycan loss was more severe in the medial plateau and in the 12-month sample (Figure 1). Safranin O staining revealed an increase in cartilage damage and Mankin grade corresponded to a decrease in PGs staining (Figure 2). Compared to the six-month old samples, there was a significant decrease in chondrocyte number in both medial and lateral tibial plateau of 12-month old samples (Figure 3).

In Situ Distribution of IGF-1

In situ hybridization of IGF-1 using single stranded DIG-UTP-labeled sense and antisense riboprobe demonstrated decreased IGF-1 levels in a temporal and spatial -dependent manner. The primary localization of IGF-1 is within the superficial zone of cartilage. The extent of IGF-1 loss was more severe in the medial plateau and in the 12-month sample. Liver tissue served as the positive control and sense probe served as the negative control for IGF-1 hybridization (Figure 4).

IGF-1 mRNA Levels

Real-time PCR demonstrated significantly decreased levels of IGF-1 mRNA in 12-month old cartilage samples compared to 6-month old samples. Results from Real-time PCR confirmed this finding, demonstrating that the level of IGF-1 mRNA in the 12-month old cartilage sample was five times less compared to levels found in the 6-month old sample. 18S served as the internal control (Figure 5).

Discussion

The exact pathogenesis of osteoarthritis has not been clearly defined. However, a combination of mechanical and biochemical factors play significant roles in the development of the disease. Increasing age and mechanical wear have been linked to osteoarthritic changes in cartilage. Scholars have focused on the role of biochemical factors, such as cytokines, chemokines, and growth factors in osteoarthritis. Inflammatory cytokines such as IL-1 and TNF-a have been associated with osteoarthritis. Chemokine molecules such as SDF-1 stimulate MMP-mediated cartilage degradation[22,23]. There is a balance between anabolic and catabolic factors that help maintain cartilage homeostasis. Chondrocyte is the only cell to maintain the balance in the cartilage. Insulin growth factor-1 plays a critical role in cartilage anabolism and has been associated with production of cartilage matrix proteins[24] and cell proliferation[25]. However, there is no directive correlation between IGF-1 and OA cartilage degradation. In this study, we show a decrease of IGF-1 level is associated with loss of chondrocyte and PGs and cartilage damage.

With the onset of cartilage damage, reparative processes are initiated, as chondrocytes begin to synthesize components that are used to rebuild the matrix cell proliferation[26,27]. This may explain why IGF-1 levels are initially elevated in synovial[28,29]. The chondrocytes, in response to increasing damage, produce more IGF-1 to augment the production of matrix proteins that are essential to the reparative process. IGF-1 induces the synthesis of collagen II and proteoglycans[24], two components integral to cartilage structure and function. However, if sufficient cartilage loss has occurred and IGF-1 levels are decreased, the chondrocyte's reparative abilities are reduced. The rate of cartilage destruction outpaces the repair, leading to the progression of osteoarthritis, while there is significant *in vitro* data supporting the protective role of IGF-1 in chondrocyte survive and proliferation[25]. Okadaic acid (OA)-induced apoptosis was significantly decreased by pre-treatment with 10 ng/ml of IGF-1 for 24 h[30]. However, the studies demonstrating *in vivo* evidence are limited. We have used the Hartley guinea pig because it is a consistent animal model for osteoarthritis[31]. Osteoarthritic changes become more pronounced in older animals[32]. Furthermore, the loading forces at the knee predispose the medial compartment to earlier and more severe degenerative changes[33].

In this study, we have demonstrated a clear association between the onset and severity of osteoarthritis with IGF-1 distribution and level in the local cartilage environment. Our morphologic analysis confirms that the medial compartment of the knee is more severely affected in osteoarthritis, with significant loss of chondrocytes, PGs, matrix, and overall structure (Figure 1 and 3). We also demonstrate that the severity of degeneration increases with age (Figure 2).

To illustrate the association between severity of osteoarthritis and IGF-1 levels, we have utilized *in situ* hybridization techniques to identify *in vivo* expression of IGF-1. Because the liver is the primary source of IGF-1 synthesis, we used it as our positive control (Figure 4A). We demonstrate decreased IGF-1 levels in the medial compartment of knee cartilage and in the 12-month cartilage samples. With increasing severity of osteoarthritis and subsequent loss of chondrocytes, PGs, and matrix integrity, a decline in IGF-1 production is observed (Figure 4B). This absolute loss of IGF-1 is associated with diminished IGF-1 production, as the expression of IGF-1 mRNA is reduced in the 12-month old sample (Figure 5). As a result, the surviving chondrocytes are less capable of synthesizing important matrix components that can adequately repair the damage. This is consistent with other lab's result. In a rat genetic mutation model, IGF-1 deficiency causes an increased severity of articular cartilage lesions of OA[34]. The compromised structural integrity of the cartilage matrix reduces its functional ability to handle increasing mechanical stress. This forms a destructive cycle that allows the continued and complete destruction of articular cartilage in osteoarthritis.

Several studies[35] have demonstrated an age-dependent increase in IGFBP-3 expression in osteoarthritic cartilage. Although it is possible that the decrease in IGF-1 distribution in the medial plateau of 12-month old cartilage is attributed to increased IGFBP-3, we demonstrate an independent decrease in the synthesis of IGF-1 by chondrocytes that exacerbates the relative decrease in free IGF-1 levels in cartilage.

Conclusions

The pathogenesis of osteoarthritis centers on mechanical and biochemical factors. Its progression is dependent upon the balance between catabolic and anabolic processes. IGF-1 plays an important and anabolic role; therefore, it serves as a protective role in osteoarthritis. We provide *in vivo* evidence that IGF-1 reduces the loss of chondrocytes and matrix integrity and is a viable target for novel therapeutic options for the management of osteoarthritis.

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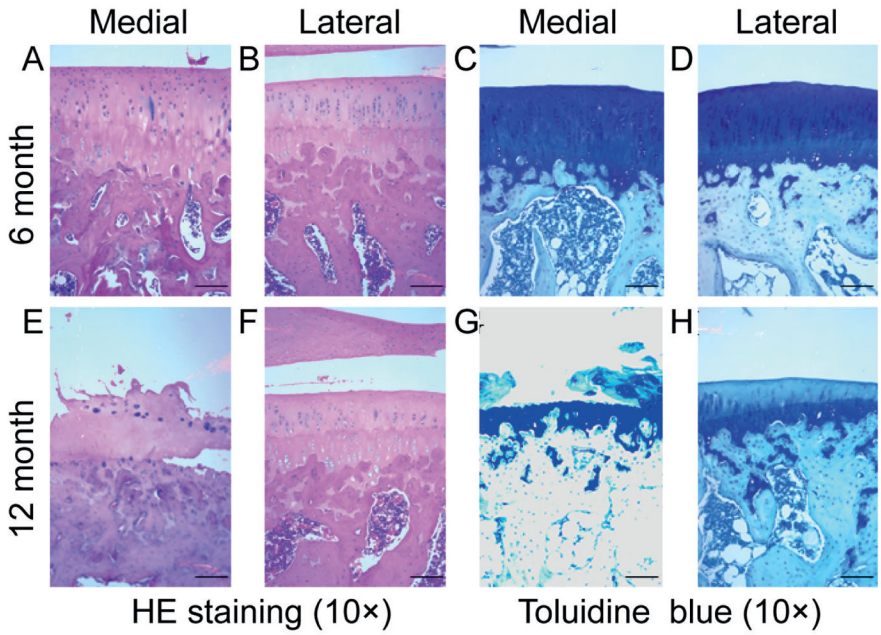

Figure 1.

Histological analysis of 6 and 12 months cartilage. HE and toludine blue staining was used to assess chondrocyte and proteoglycan loss. ***(A-B)***: There is minimal cell and matrix disruption in the medial and lateral plateau of the 6 months cartilage. ***(E-H)***: In the medial plateau of the 12 months cartilage, there is a cartilage destruction with the crack between calcified and uncalcified cartilage interface, chondrocyte loss, and matrix breakdown with loss of proteoglycans. Even with the intact articular surface, there is a loss of chondrocytes and matrix components in the lateral plateau of 12 month samples. Scale bar = 200 μm.

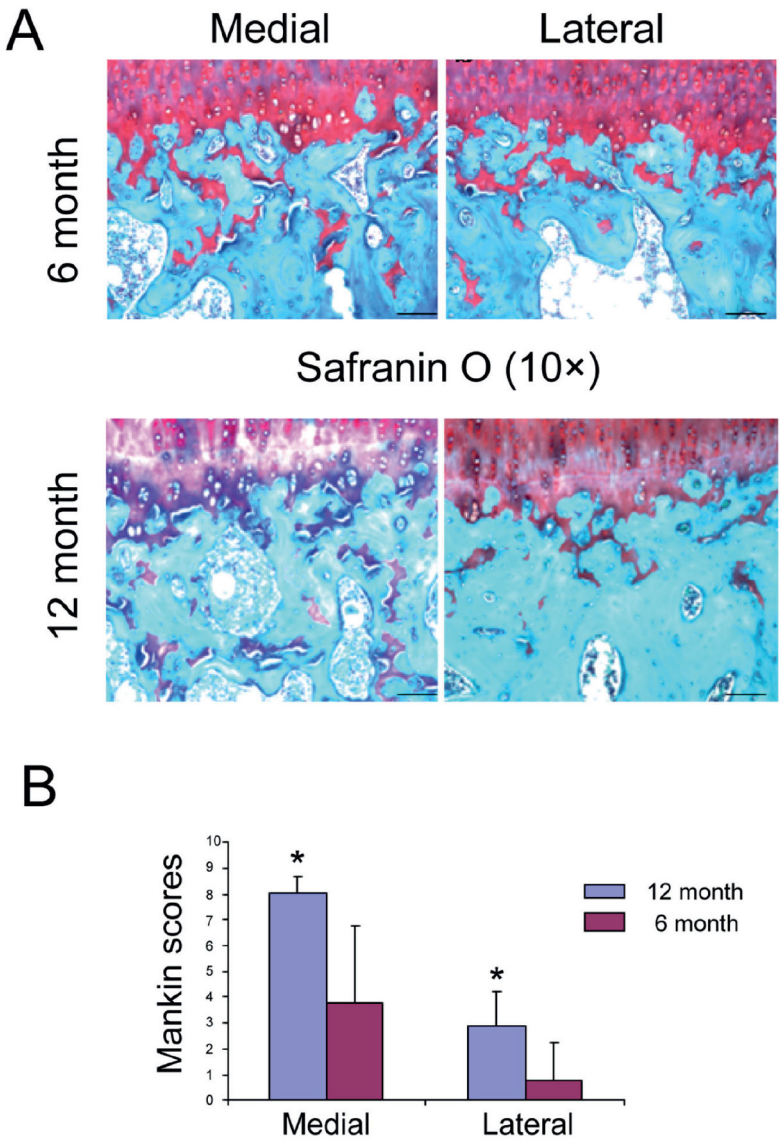

Figure 2.

Safranin O staining and Mankin grading ***(A)*** Safranin O staining and ***(B)*** the modified Mankin score showed a marked increase in cartilage damage for the 12 month knees at the medial side as compared to the 6-month joints. Loss of proteoglycan staining and chondrocytes and cartilage destruction were evident in the 12-month-old animals. Scale bar = 200 μm.

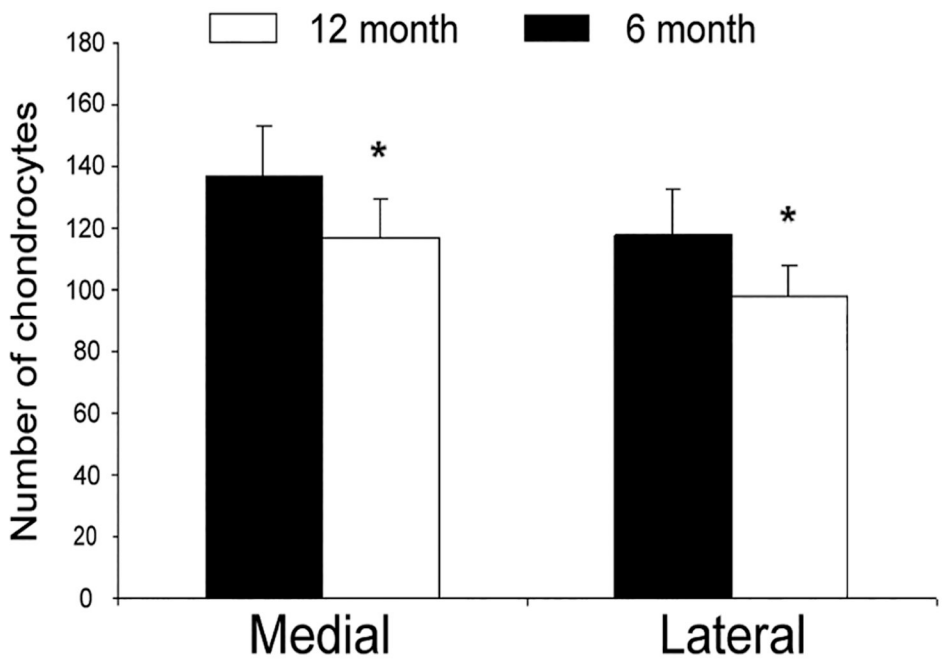

Figure 3.

Quantification of chondrocytes in 6 and 12 month cartilage. Light microscopy was used to count the number of chondrocytes in 6 and 12-month cartilage samples. The number of chondrocytes was higher in the 6 month cartilage, with loss of cellularity observed in the 12 month cartilage. This pattern was observed in both the medial and lateral plateau. Bar graphs show the averages of quantified data from 8 animals. *: Statistically significant in comparison to 6-month-old animals, $p<0.05$.

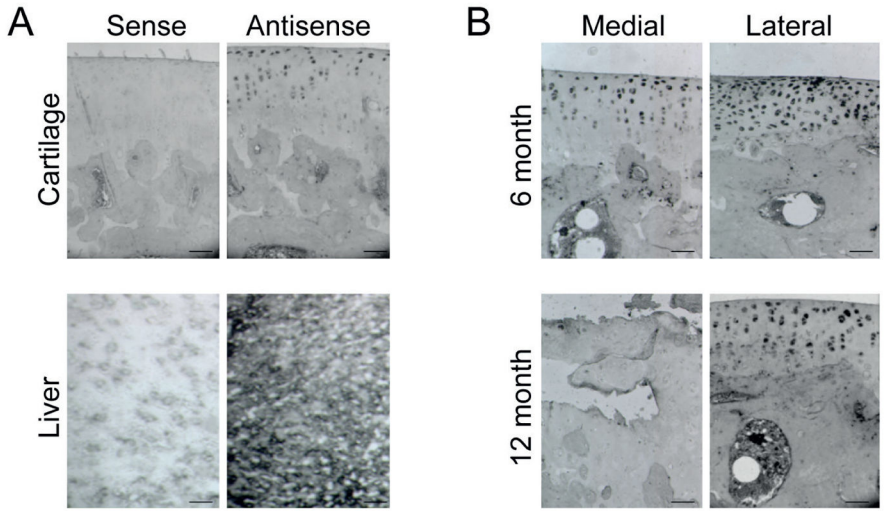

Figure 4.

In situ distribution of IGF-1: ***A,*** Positive and Negative control of DIG-UTP IGF-1 insitu hybridization. Negative and positive controls of *in situ* hybridization on paraffin embedded sections of cartilage and frozen section of liver with single-stranded DIG-UTP-labeled sense and antisense riboprobes; ***B,*** *In situ* hybridization of IGF-1 in 6 and 12 month cartilage. The distribution of IGF-1 was detected with *in situ* hybridization on paraffin embedded sections with single-stranded DIG-UTP-labelled antisense riboprobe. IGF-1 was distributed mainly in uncalcified cartilage. In the 6 month sample, IGF-1 is expressed in the superficial cartilage and that its expression was more pronounced in the lateral plateau of cartilage. In the 12 month sample, there is obvious cartilage destruction with minimal IGF-1 expression in the medial plateau. In the same sample, the cartilage structure is intact in the lateral plateau, but there is decreased IGF-1 expression when compared to the 6month lateral plateau. Scale bar = 100 μm.

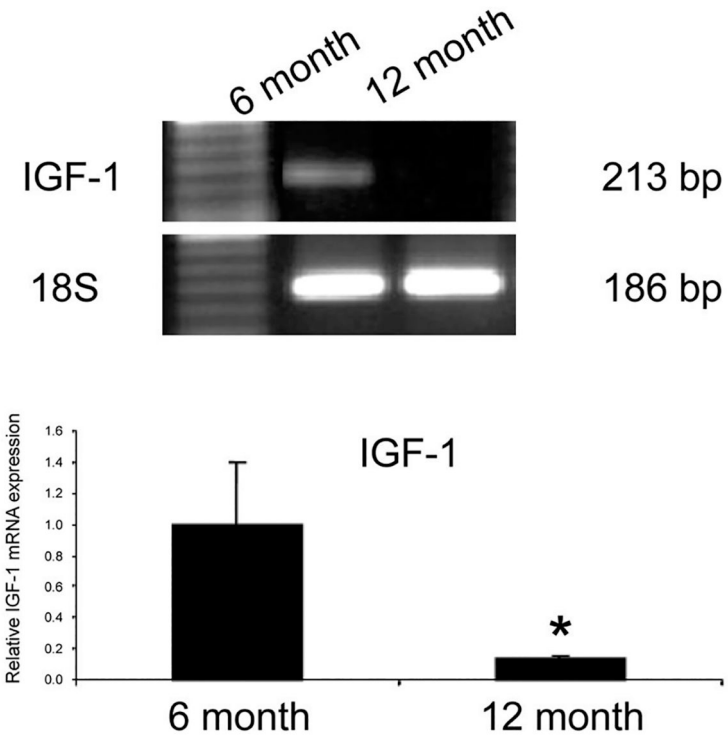

Figure 5.

mRNA levels of IGF-1 in 6 and 12 month cartilage: Real-time PCR was used to quantify mRNA levels of IGF-1 in 6 and 12 month old cartilage. Using 18S as the internal control, there was decreased IGF-1 mRNA in 12 month old sample. Bar graphs show the averages of quantified data of IGF-1 using Real-time PCR from three independent experiments. *: Significant difference in comparison to 6-month-old animals, $p<0.05$.