Local administration of growth hormone stimulates tendon collagen synthesis in elderly men

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The mechanical properties of tendon and ligaments depend primarily on orientation and density of collagen fibrils as well as on intra- and intermolecular cross-links (19). Cross-sectional observations have shown a reduced total collagen content in elderly individuals, whereas but a higher content of enzymatic and nonenzymatic cross-links has been observed in tendon of elderly individuals compared with young men (8). These observations may indicate a reduced tendon collagen turnover in elderly individuals.

It is well known that there is an age-associated decline in the activity of the growth hormone (GH)-insulin-like growth factor I (IGF-I) system (22, 31). Several observations indicate that the GH-IGF-I system plays an important regulatory role in the muscular skeletal system. In tendon and ligament explants, IGF-I stimulates fibroblast proliferation and synthesis of extracellular matrix proteins (e.g., collagen; Refs. 1–3, 10, 26). Furthermore, IGF-I injection promotes tendon and ligament healing in animals after collagenase-induced tissue atrophy (9) or ligament disruption (21, 30). In patients with acromegaly and GH deficiency, local muscle IGF-I mRNA expression correlates positively with muscle type I collagen mRNA expression (12). Furthermore, in young healthy individuals, 14 days of systemic rhGH administration increases circulating IGF-I and local expression of IGF-I in tendons, accompanied with a rise in tendon collagen mRNA expression and tendon collagen synthesis (11). Finally, it has recently been demonstrated that local injections of IGF-I into the patella tendon increase tendon collagen synthesis in healthy elderly individuals (14). An interesting question is, whether local injection of GH, without triggering synthesis and secretion of hepatic IGF-I, is able to enhance tendon collagen synthesis either directly or indirectly by enhancing the local IGF-I production. Therefore, the aim of the present study was to examine whether local injections of rhGH into the patellar tendon enhance tendon collagen synthesis in elderly men.

MATERIALS AND METHODS

Subjects

A total of 12 healthy men were enrolled in the study. Subject characteristics were (means ± SE): age: 61 ± 1 yr (range 55–69 yr); weight: 88 ± 4 kg; height: 181 ± 2 cm; body mass index: 27 ± 1 kg/m²; and lean body mass: 64 ± 2 kg. The local Ethics Committee for Medical Research in Copenhagen (H-2–2010-039) approved the protocol. Before inclusion, informed written and oral consent was obtained from each subject according to the Declaration of Helsinki. Exclusion criteria for participation in the study were diabetes, obesity (body mass index >30 kg/m²), smoking, skeletal muscle diseases, hypermobility, previous or present knee injuries or patellar tendinopathy, use of medicine with a potential effect on connective tissue, and screening blood sample values outside normal range.

Health Examination and Screening

Each subject underwent a medical evaluation (including routine blood tests) and measurement of body composition. One to three weeks before the experimental days the subjects underwent health examination and screening procedures, which included questionnaires about disease and training history, blood samples, and measurements about disease and training history, blood samples, and measurements.
of body composition (height, weight and dual-emission X-ray absorptiometry). Blood samples were analyzed for plasma concentrations of creatinine, creatinine kinase, calcium, C-reactive protein, alkaline phosphatase, aspartate aminotransferase, alanine transaminase, erythrocyte volume fraction, sodium, potassium, blood concentrations of hemoglobin (Hb), thrombocytes, leucocytes, and HbA1c and serum concentrations of thyroid stimulating hormone.

Study Design

The study was designed as a placebo-controlled, double-blinded study comparing effects of two local injections of either rhGH or saline (control) on tendon collagen synthesis. In the morning at day 1 of the experiment, rhGH was injected into the patellar tendon of one leg, and a similar volume of saline was injected into the contralateral patellar tendon. The following morning the procedures were repeated. During the subsequent hour, tendon collagen synthesis was measured directly by stable isotope incorporation and indirectly by microdialysis technique. An overview of the design of the experiment is illustrated in Fig. 1.

Experimental Days

On each experimental day, each subject attended the laboratory at 0800 after an overnight fast. No strenuous physical exercise was allowed for the 2 days before the experimental days. This was to avoid that the mechanical loading caused stimulation of tendon collagen synthesis above the stimulus from normal everyday activities. Each subject was instructed to continue normal dietary habits while enrolled in the study period.

GH and Saline Injections

The two injections of rhGH (each 0.1 mg rhGH Genotropin MiniQuick) into the patellar tendon were performed randomly between subjects into either the dominant or nondominant leg. A similar volume (125 µl) of saline (control) was injected into the contralateral patellar tendon. The injections were performed under ultrasound guidance 1 cm distal from the distal patella tip. At day 1 of the experiment, the subjects rested in bed for 1 h after the rhGH and saline injections to minimize disappearance of the infused rhGH from the tendon area. The following day, the rhGH and saline injections were repeated in the same leg, respectively, as on the previous day. After the second bilateral injections, the subjects stayed in bed and fasted until the end of the experiment. Subjects were allowed to drink water throughout the experimental period.

Tendon Collagen Fractional Synthesis Rate Measured by Stable Isotope Technique

A primed continuous infusion of L-[ring-13C6]phenylalanine was initiated 30 min after the last rhGH and saline injections for measurements of patellar tendon collagen fractional synthesis rate (FSR) in both legs. The tracer infusion was maintained until the completion of the study ~6½ h later. The primer was intravenously infused over a period of 2 min and contained 200 mg L-[ring-13C6]phenylalanine (>99 Atoms% 13C-proline; Cambridge Isotope Laboratories, Andover, MA) and 2,000 µg unlabeled phenylalanine dissolved in 0.9% NaCl using sterile techniques. Thereafter, the continuous infusion of L-[ring-13C6]phenylalanine (6 µmol·kg LBMM⁻¹·h⁻¹) was initiated. To analyze for isotopic enrichment (tracer-to-tracee ratio) blood samples were drawn before (background tracer enrichment) and +10, 20, 30, 60, 120, 180, 240, 300, and 360 min after the initiation of the primer. Then, tendon biopsies were obtained from the patella tendon (~10 mg) of each leg after a sterile wash of the skin and anesthetizing the skin at incision site with 1% lidocaine. Subsequently, a 3- to 5-mm long skin incision was created using a scalpel. The biopsies were obtained using a Bard MAGNUM Biopsy Instrument (C. R. Bard, Covington, GA) with a 14-gauge disposable core biopsy needle under ultrasound guidance. The biopsy needle was inserted through this incision onto the tendon surface at an ~30° angle. Biopsies were cleaned from adipose tissue and blood under microscope, frozen in liquid nitrogen, and stored at ~80°C for subsequent analysis.

Plasma phenylalanine enrichment. Plasma for determination of phenylalanine enrichment was prepared as previously described (4, 32) and analyzed as its N-butyldimethylsilyl derivative by gas-chromatography mass-spectrometry (GC, Trace GC 2000 series, MS, Automass Multi; Thermo Quest Finnigan, Paris, France) and separated by a capillary column (CP-SIL 8, CB low bleed, 30 m × 0.32 mm, coating 0.25-µm column, Chrompack, Varian, Palo Alto, CA). The plasma phenylalanine enrichment values measured during the infusion protocol are illustrated in Fig. 2. The phenylalanine enrichment from 20 to 60 min after start of the infusion protocol was significantly different from the period from 180 to 360 min (Friedman repeated measures ANOVA on ranks, P < 0.05). This may influence the calculated absolute FSR values. Nevertheless, the focus in the present study was to determine the difference in incorporation rate of the labeled phenylalanine between the GH and control leg.

Tendon preparation. Details regarding this procedure have been provided elsewhere (5, 25). Briefly, tendon (5–10 mg) biopsies were homogenized in buffer (0.15 M NaCl, 0.1% Triton X-100, 0.02 M Tris·HCl, and 5 mM EDTA, pH 7.4) and centrifuged with 1,600 g and the pellet was subsequently washed with 0.7 M KCl, centrifuged with 1,600 g, and hydrolyzed in 6 M HCl at 110°C overnight. Then, amino acids were extracted through disposable columns using acidic cation exchange resin ( Dowex AG-50W; Bio-Rad, Sundbyberg, Sweden). The amino acids were derivatized as their N-acetyl-n-propyl esters (24), and the 13C abundance under the phenylalanine peak was determined by gas chromatograph combustion isotope ratio mass spectrometry (Delta Plus XL; Thermo Finnigan, Bremen, Germany).
Fig. 2. Plasma enrichment of ring-[13C6]phenylalanine during a primed, constant infusion of ring-[13C6]phenylalanine. Unit of atom percent excess (APE) specifies the level of isotopic abundance above a background reading.

using a column CP-Sil 19 CB 60 m × 0.32 mm, coating 0.25 μm (ChromPack, Varian, Palo Alto, CA).

Calculations. The tendon collagen FSR was calculated based on the incorporation of tracer (l-[ring-13C6]phenylalanine) into tendon tissue proteins, using a standard precursor product model: FSR (\% h⁻¹) = \( \Delta F_{\text{product}} \times E_{\text{Precursor}}^{-1} \times \Delta t (\text{h}) \times \text{atom dilution factor} \times 100\% \), where \( \Delta F_{\text{product}} \) is the difference in tracer enrichment between the tendon tissue samples and the baseline plasma tracer enrichment. \( E_{\text{Precursor}} \) is the constant tracer enrichment measured in plasma in the time period from the initiation of the priming and the final biopsy (\( \Delta t \)). The atom dilution factor is 14/6.

Tendon Collagen Synthesis Measured by Microdialysis

The microdialysis protocol was initiated 30 min after the last rhGH/saline injections. After initial preparation of incision sites with local anesthetic (lidocaine 1%) ethylene oxide sterilized catheters with high molecular mass cut-off (3,000 kDa; membrane length of 30 mm and inner diameter of 0.50 mm) were inserted under ultrasound guidance in the peritendinous spaces of patellar tendons as previously described (23). The inflow-tube of the microdialysis catheter was connected to a high-precision syringe pump with infusion rate of 2 μl/min. The outflow-tube, and after 90 min perfusion of the catheter, dialysate was collected in four 1-h periods and stored at −80°C until the analyses were performed. The microdialysis catheters were inserted in the last 10 subjects because catheters were not available at the beginning of the experiment. Dialysate collected from the interstitial fluid surrounding the patellar tendon was analyzed for a marker of type I collagen synthesis, the amino terminal propeptide of type I collagen (PINP) as previously described in detail (23). Dialysate collected within 3–4 h after the last injections was used for PINP analyses performed by the use of a sandwich ELISA utilizing purified alpha 1-chain specific rabbit antibodies (donated by Teisner B, Department of Medical Microbiology, University of Southern Denmark, Odense, Denmark; Ref. 17). The coefficient of variation (CV; double determination) was 1.0 ± 0.2% on average.

GH and IGF-I Concentrations

Blood samples for analyses of serum GH and IGF-I were taken from a cubital vein into sealed vials at baseline, 30 min and 6½ h after the last injections. After separation by centrifugation, serum was stored at −80°C until analysis. Serum GH and tendon dialysate GH were determined by TR-IFMA after acid-ethanol extraction, as previously described (13). All samples were measured in the same assay run (intra assay CV% <5%). Serum IGF-I and tendon dialysate IGF-I were determined by TR-IFMA after acid-ethanol extraction, as previously described (13). All samples were measured in the same assay run (intra assay CV% <5%).

Dialysate collected within 2–3 h and within 5–6 h after the last injections was pooled and analyzed for IGF-I bioactivity. IGF-I bioactivity in the tendon dialysate and serum was measured using an in house IGF-I kinase receptor activation assay (KIRA) based on cells transfected with the human IGF-IR gene, in accordance with the method described by Chen et al. (7). The bioassay was sensitive (detection limit of 0.08 μg/l), specific (cross-reactivity of insulin, insulin analogs, and proinsulin was <1%; IGF-II cross-reactivity was 12%), and accurate (within- and-between-assay CV <7 and <15%).

Statistical Analysis

One-way repeated-measures ANOVA was used to test for changes in serum IGF-I from baseline to the end of the experimental day. A Friedman repeated-measures ANOVA on ranks was used to test for changes in serum GH from baseline to the end of the experimental day and for analysis of changes in isotope enrichment during the continuous infusion of labeled phenylalanine. Nonparametric statistics was used to test for difference in tendon collagen FSR (Wilcoxon signed-rank test) between the GH and control leg since data was not normally distributed. Parametric statistics (paired Student’s t-test, one-tailed) were used to test for difference between the legs in tendon collagen synthesis (PINP) and bioactive IGF-I in the microdialysis dialysate. The level of significance was set at \( P = 0.05 \).

RESULTS

The indirect marker for type I collagen synthesis, [PINP] in the peritendinous tissue, was enhanced from 3 to 4 h after the last injections in the GH leg compared with the control leg (means ± SD, 18 ± 3 vs.12 ± 3 μg/l; \( P = 0.05 \); Fig. 3). Further, tendon collagen FSR tended to be higher in the GH leg (median, 25–75%: 0.008% h⁻¹, 0.003–0.016% h⁻¹) compared with the control leg (0.004% h⁻¹, 0.002–0.008% h⁻¹) in the 6½-h period after the last injections (\( P = 0.08 \); Fig. 3). The increase in tendon FSR after rhGH injections was evident in 10 out of 12 subjects.

Serum GH increased significantly 30 min after the local injections (\( P < 0.05 \)) but did not differ from baseline when determined 6½ h after the injections (Fig. 4). Serum IGF-I did not change during the experiments (\( P = 0.20 \); Fig. 4). IGF-I bioactivity in the dialysate from the peritendinous region in front of the patellar tendon was higher in the GH leg than in the control leg (Fig. 5). Circulating IGF-I bioactivity was higher than local IGF-I bioactivity, but was not different 6½ h after the last injections (\( P = 0.12 \); Fig. 5).

DISCUSSION

The effect of local rhGH injections directly into the human tendon has not been measured previously. In the present study, we observed an increase in tendon collagen FSR in 10 out of 12 subjects (\( P = 0.08 \)). Furthermore, the indirect marker for type I collagen synthesis collected in the peritendinous fluid was significantly higher after rhGH injections. This was associated with a significantly higher IGF-I in vitro bioactivity in the dialysate from the peritendinous region. Taken together, our data suggest that GH exerts direct stimulatory effects on tendinous collagen synthesis, which involves local IGF-I production.
Tendon structure and biomechanical properties influence overall function of the tendon and skeletal muscle complex. An increased understanding of the regulation of tendon and ligament collagen turnover is of importance in future injury prevention and rehabilitation after tendon and ligament injuries. The present findings add to the knowledge regarding the importance of GH in the regulation of tendon collagen synthesis. Furthermore, our data may give rise to development of new treatment strategies.

In the present study, elderly subjects were included. Characteristics for elderly individuals include lower content of the most common structural protein in tendons, collagen (8), reduced magnetic resonance imaging tendon signal intensities, and increases in tendon cross-links. These changes have in some, but not all human studies (6, 8), been related to less extensible tendon structures (20). The present results suggest that the changes in tendon collagen structure associated with aging are related to the progressive decline in 24 h GH secretion rates (37) and thereby reduced bioavailability of IGF-I. Thereby, the age-associated decline in the activity of the GH-IGF-I system (22, 31) may not only be linked to the molecular, cellular and functional changes that occur during aging in the cardiovascular system (33, 35). The presence of these hormones locally in the peripheral connective tissues such as the tendon and ligament seems to be of importance for the maintenance of the structural proteins within these tissues.

The size of the enhancement of the synthesis rate in response to rhGH administration was variable between subjects. Several challenges could have caused this difference in response. This could be due to difficulties in obtaining the tendon biopsy exactly at the injection site when measuring tendon collagen FSR as well as in placing the microdialysis catheters close to the injection site, thereby causing differences in the diffusion distance between tendon and the microdialysis membrane. Nevertheless, the present tendency towards a significantly higher tendon collagen synthesis rate after rhGH injections, measured by two methodological approaches, supports a stimulating effect of local rhGH injections in human connective tissue; using stable isotopes to determine changes in the tendon tissue, and simultaneously using the microdialysis technique to evaluate collagen synthesis peritendinously (23). Furthermore, the baseline level of GH and IGF-I may be of importance for the response in collagen synthesis. The subject with the highest circulating GH and IGF-I concentrations before the last injections and during the whole experimental day was also characterized by the highest tendon collagen FSR in the control leg. Further, this patient showed no increase in tendon FSR and tendon PINP following local injection with GH stimulation; in fact a numeric lower tendon collagen synthesis was observed.

![Collagen synthesis](image1)

**Fig. 3.** A: difference in tendon collagen fractional synthesis rate (FSR) in the patellar tendon of the leg exposed to rhGH injections compared with the control tendon (n = 12 paired-samples; ΔFSR). Tendency towards a difference in tendon FSR between the control and GH tendon, P = 0.08. B: difference in amino terminal propeptide of type I collagen (PINP) in the peritendinous fluid of the leg exposed to rhGH injections compared with the control (n = 10 paired-samples) measured within 3 to 4 h after the last injections (ΔPINP). Significant difference in tendon PINP between the control and GH tendon, P = 0.05.

![Serum GH](image2)

**Fig. 4.** Serum (s)-GH (A; ng/l) and serum insulin-like growth factor I (B; IGF-I; μg/l) at baseline and half hour and 6½ h after the last injections of rhGH and saline at day two of the experiment. *P < 0.05, significant difference in serum GH between baseline and one-half hour.
This suggests that tendon collagen synthesis in this subject was already maximally stimulated by endogenous GH.

This is the first time IGF-I bioactivity is measured in tendon dialysate and is encouraging for further investigations of the regulation of IGF-I bioactivity in the extracellular matrix tissues in general. In the present study, we observed significantly higher concentration of bioactive IGF-I in the tendon dialysate collected from the peritendinous space in front of the patella tendon of the GH leg compared with the control leg. These findings indicate that local injections of GH stimulate local synthesis of IGF-I rather than an obligatory release of IGF-I from the liver and thereby enhance IGF-I action in tendons. As recently observed, the higher concentration of bioactive IGF-I in the GH-leg may partly explain the higher tendon collagen synthesis after local rhGH injections. In support, local injections of IGF-I itself into the tendon have been reported to increase tendon collagen synthesis markedly (14). The local increase in bioactive IGF-I after the GH injection is probably only transient. Furthermore, the effect of repetitive GH injections may be reduced by time since stimulation of the synthesis of IGF-I binding proteins may reduce the local increase in bioactive IGF-I and thereby the increase in tendon collagen synthesis, which is not related to a direct effect of GH.

After systemic rhGH administration for 14 days increases in circulating IGF-I levels and the rise in local expression of IGF-I in tendons were accompanied by a rise in tendon collagen mRNA expression and tendon collagen synthesis in young healthy individuals (11). In patients with acromegaly or GH deficiency, positive correlations between local IGF-Ie mRNA expression and muscle collagen I and III mRNA expression have been observed (12). In contrast, no change in collagen synthesis in tendon and skeletal muscle was observed after 2 wk of GH receptor blocker supplementation (Pegvisomant) in a randomized placebo controlled study using healthy individuals (27). However, in the latter study the decrease in serum IGF-I was only moderate (20%; Ref. 27). We aimed to investigate whether it was possible to stimulate tendon collagen synthesis by direct injections of rhGH into the tendon without enhancing the systemic levels of GH and IGF. Apart from a short-term and very moderate increase in circulating GH this was achieved. It is important to note that the small rise in circulating GH did not result in any rise in circulating IGF-I. Nevertheless, in the present study, bioactive IGF-I measured in the peritendinous fluid was enhanced \((P = 0.05)\). Our data suggest that local injection of rhGH may either stimulate local tendon synthesis directly and/or indirectly by stimulating local IGF-I. A stimulating effect of local IGF-I administration on collagen synthesis is in agreement with previous results from in vitro and experimental in vivo studies (10, 34). In animals, the response to mechanical loading includes increased levels of circulating GH and IGF-I, findings that have been coupled to a simultaneous increase in tendon collagen expression (15, 29).

Nevertheless, the present results suggest that the exercise increase in GH triggered by exercise may directly stimulate tendon collagen synthesis.

This is the first time rhGH is injected into human patellar tendons with the purpose to raise tendon collagen synthesis. Therefore, the optimal time point for measurement of tendon collagen synthesis in response to injection of GH was unknown. After acute exercise, we observed an increase in tendon collagen FSR already a few hours after exercise, peaking 24 h postexercise (25). Thus similarly there may be a time lag from the injection of rhGH is undertaken until a stimulating effect on tendon collagen FSR detected. Assuming the effect of GH injection mimics the exercise-induced stimulation of tendon collagen FSR, one injection of rhGH 24 h before measurements of tendon collagen FSR would be the most appropriate. Nevertheless, the response time might also be shorter. Thus, to reduce the risk of overseeing a positive effect of rhGH administration, we chose to administrate two injections of rhGH; one at 24 h and a second one-half hour before the 6-h tendon collagen FSR measurement period.

We aimed to inject a high dose of rhGH to improve the possibility for detecting a stimulating effect of GH on tendon collagen synthesis. At the same time, we aimed to avoid an increase in circulating GH levels in order not to 1) stimulate the hepatic IGF-I secretion and 2) transfer the effect of the rhGH injection to the contralateral control leg. The average daily GH production at age 20 yr is \(\approx 500 \, \mu g\), declining \(\sim 14\%\) per decade (16). The recommended dose of rhGH to GH deficient adults is up to \(80 \, \mu g\cdot kg^{-1}\cdot wk^{-1}\). In light of these facts, we aimed to reach a local GH concentration of \(100 \, \mu g/l\) in the...
knee region. With the assumption that the knee region corresponds to a compartment of 1 l, 100 μg of rhGH (0.125 ml, 800 μg/ml) was injected.

In the present study, the injection of rhGH was placed in the mid-substance of the patella tendon, and ultrasonography assisted the guiding of the needle to ensure placement of the substance. Evidently, it is difficult to document exactly how much of the injected volume that may diffuse into the surrounding tissue, but from a recent cadaver study it is likely that the injected substance will spread proximally and distally along the loose connective tissue between fascicles (36). Furthermore, it cannot be excluded that part of the injected rhGH will disappear via the injection canal into the circulation, which would underestimate the effect of the GH injection. Nevertheless, no marked change in circulating IGF-I was noted in any of the subjects during the experimental period to support a systemic uptake of the injected GH. Furthermore, the one subject who experienced a transient increase in circulating GH 30 min after the injections experienced no change in circulating IGF-I but a markedly higher tendon collagen synthesis rate in the GH leg compared with the control leg. Still, we cannot exclude that the injected rhGH may have diffused into the surrounding tissue in some of the subjects and thereby reduced the response in tendon collagen synthesis.

In conclusion, local rhGH injections into the human patellar tendon stimulate tendon collagen synthesis either directly or indirectly by enhancing bioactive IGF-I. In vitro (1–3, 10, 26) and animal findings (9, 21, 30) suggest that growth factors such as IGF-I may optimize and accelerate tissue healing and shorten time to functional recovery. Systemic administration of GH is associated with side effects. Since the present results indicate that local GH within the connective tissue plays an important physiological role in the regulation of collagen-rich tissues, local GH administration may be an useful means to improve healing in patients during rehabilitation of tendon and ligament injuries and posturgery. Especially elderly people characterized by an age-dependent decline in the GH/IGF-I activity may benefit from local GH administration during rehabilitation of tendon and ligaments. However, further investigations are needed to test this hypothesis.

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