

# Growth hormone stimulates the collagen synthesis in human tendon and skeletal muscle without affecting myofibrillar protein synthesis

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In skeletal muscle and tendon the extracellular matrix confers important tensile properties and is crucially important for tissue regeneration after injury. Musculoskeletal tissue adaptation is influenced by mechanical loading, which modulates the availability of growth factors, including growth hormone (GH) and insulin-like growth factor-I (IGF-I), which may be of key importance. To test the hypothesis that GH promotes matrix collagen synthesis in musculotendinous tissue, we investigated the effects of 14 day administration of 33–50  $\mu\text{g kg}^{-1} \text{day}^{-1}$  recombinant human GH (rhGH) in healthy young individuals. rhGH administration caused an increase in serum GH, serum IGF-I, and IGF-I mRNA expression in tendon and muscle. Tendon collagen I mRNA expression and tendon collagen protein synthesis increased by 3.9-fold and 1.3-fold, respectively ( $P < 0.01$  and  $P = 0.02$ ), and muscle collagen I mRNA expression and muscle collagen protein synthesis increased by 2.3-fold and 5.8-fold, respectively ( $P < 0.01$  and  $P = 0.06$ ). Myofibrillar protein synthesis was unaffected by elevation of GH and IGF-I. Moderate exercise did not enhance the effects of GH manipulation. Thus, increased GH availability stimulates matrix collagen synthesis in skeletal muscle and tendon, but without any effect upon myofibrillar protein synthesis. The results suggest that GH is more important in strengthening the matrix tissue than for muscle cell hypertrophy in adult human musculotendinous tissue.

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**Abbreviations** 1 RM, 1 repetition maximum; AUC, area under the curve; cDNA, complementary DNA; CI, confidence interval; COL1A1, collagen  $\alpha 1$  (I); COL3A1, collagen  $\alpha 1$  (III);  $C_t$ , threshold cycle; FSR, fractional synthesis rate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GC-C-IRMS, gas-chromatography-combustion-isotope-ratio-mass-spectrometer; GH, growth hormone; GHD, growth hormone deficient; IGF-I, insulin-like growth factor-I; IGF-IEa, insulin-like growth factor-IEa; IGF-IEc, insulin-like growth factor-IEc; IGFBP, IGF-binding protein; NAP, *N*-acetyl-*N*-propyl; PIIP, pro-collagen III pro-peptide; rhGH, recombinant human GH; RPLP0, large ribosomal protein P0; sGH, serum GH; sIGF-I, serum IGF-IEa; sIGFBP, serum IGFBP.

## Introduction

Locomotive and supportive functions are dependent upon the transmission from muscle to the skeleton of force via intramuscular connective tissue and tendon. Collagen is

the most important protein in conferring the required mechanical properties (Kjaer, 2004). The adaptation of matrix tissue to mechanical load is probably mediated *inter alia* by growth factors, of which growth hormone (GH) and insulin-like growth factor-I (IGF-I) are among

the most important. IGF-I has a well characterized muscle growth-promoting effect in loaded animal muscle due both to circulating IGF-I secreted by the liver under GH control and after local production of a number of IGF-I variants (Goldspink, 1999; Adams *et al.* 2007). Furthermore, muscle-specific overexpression of IGF-I in transgenic mice (Musaro *et al.* 2001) and acute ectopic expression of IGF-I by electroporation result in muscle hypertrophy (Alzghoul *et al.* 2004). Although these findings suggest involvement of GH and IGF-I in activity-dependent muscle plasticity, direct evidence for such a role has never been demonstrated in adult human muscle.

In animal tendon explants there is a dose-dependent effect of IGF-I on cell proliferation and collagen synthesis (Abrahamsson *et al.* 1991; Banes *et al.* 1995; Murphy & Nixon, 1997) and cognate effects can be seen after GH treatment of various length in muscle and tendon of rats, sheep and pigs (Pell & Bates, 1987; Kyparos *et al.* 2002; Choy *et al.* 2005). In patients with acromegaly, high blood concentrations of GH and IGF-I are associated with increased thickness and content of collagen rich tissue (Gonc & Kandemir, 2007; Zgliczynski *et al.* 2007), but the dynamics of this process have not been studied in detail. In hypopituitary men, who received GH treatment, there is a consequent rise in the expression of mRNA for both IGF-I and collagen 3A1 in skeletal muscle (Sjogren *et al.* 2007), but no studies so far have directly determined the possible effects of GH/IGF-I on local collagen protein production in human musculotendinous tissue.

Mechanical loading of muscle and tendon increases expression of both IGF-I and collagen mRNA (Olesen *et al.* 2006; Heinemeier *et al.* 2007a), but no direct coupling between GH/IGF-I and collagen synthesis determined dynamically has been demonstrated after increases in loading.

Although it has been shown that recombinant human GH (rhGH) administration has no effect on human muscle size and muscle protein synthesis (Rennie, 2003) it is nevertheless commonly assumed by coaches and athletes that this is not true. A perception of increased performance of explosive events is almost certainly mistaken (Liu *et al.* 2008); however, there may be subjective changes in kinaesthesia due both to the perception of oedema and possibly increased muscle stiffness due to growth of collagen tissue. This, together with increased collagen mass observed in patients with acromegaly (Gonc & Kandemir, 2007; Zgliczynski *et al.* 2007), reinforced our interest in the effects of rhGH on collagen metabolism in the musculoskeletal system.

In the present study, we wished to test the major hypothesis that manipulating blood concentration of GH and IGF-I, as well as tissue IGF-I mRNA expression, would increase collagen mRNA expression and collagen protein synthesis in skeletal muscle and tendon. Furthermore, we

wished to check the previous report of a lack of stimulation of human muscle protein synthesis (Yarasheski *et al.* 1992) by measuring the synthesis of myofibrillar protein. We aimed to fulfill our objectives by studying the responses of healthy young men to 2 weeks of rhGH administration.

## Methods

### Healthy volunteers and GH administration

Ten healthy, sedentary men ( $30 \pm 2$  years) were recruited for the study. All were non-smoking, not taking any medication, and judged healthy based on routine medical examination and medical history. The participants gave written informed consent to a protocol adhering to the *Declaration of Helsinki*, which was approved by the Ethics Committee of Copenhagen and Frederiksberg (KF (01) 258765). The study was carried out as a double-blinded, randomized, placebo-controlled cross-over trial. rhGH (Norditropin, Novo Nordisk, Bagsvaerd, Denmark) was administered by daily subcutaneous injection in the thigh for 14 consecutive days. rhGH dosage was  $33.3 \mu\text{g kg}^{-1} \text{day}^{-1}$  on days 1–7 and  $50 \mu\text{g kg}^{-1} \text{day}^{-1}$  on days 8–14. During each supplementation period, the participants visited the laboratory twice to detect possible side effects and to ensure compliance. The two periods were separated by a 5 months washout period.

### Exercise

All participants did one bout of leg extension exercise (Technogym, Super Executive Line, Gambottola, Italy) with concentric unilateral load of the quadriceps muscle and patella tendon. Prior to the study, one-leg one repetition maximum (1 RM) was determined, and the workload was defined as  $10 \times 10$  repetitions at 70% of 1 RM.

### Tissue biopsy procedures

Biopsies were taken 24 h after exercise with the subjects in a post-absorptive state (10 h fasting). The sample sites were prepared with local anaesthetics (lidocaine, 1%), and tendon and muscle was sampled from both the exercised and the rested leg. Tendon samples were taken from the patella tendon (Bard Magnum Biopsy Instrument, C.R. Bard, Inc., Covington, GA, USA) with a 14 g needle (Movin, 2000). The total wet weight of each tendon sample was from 5 to 10 mg per biopsy. Muscle was sampled from the vastus lateralis (m. quadriceps) muscle using a 5 mm Bergström needle with suction (Bergstrom, 1975). The total wet weights of the muscle samples were from 50 to 80 mg. Tendon and muscle samples were frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$ .

**Table 1. Primers for real-time PCR**

MRNA	Sense	Antisense	GenBank Acc. No.
IGF-IEa <sup>#</sup>	GACATGCCCAAGACCCAGAAGGA	CGGTGGCATGTCACTTCTCACTC	NM_00618.3
IGF-IEc <sup>#</sup>	GCCCCATCTACCAACAAGAACAC	CGGTGGCATGTCACTTCTCACTC	NM_001111283.1
COL1A1	GGCAACAGCCGCTTCACCTAC	GCGGGAGGACTTGGTGGTTTT	Z74615.1
COL3A1	CACGGAAACACTGGTGGACAGATT	ATGCCAGCTGCACATCAAGGAC	NM_000090.3
GAPDH	CCTCTGCACCACCAACTGCTT	GAGGGGCCATCCACAGTCTTCT	NM_002046.3
RPLP0	GGAAACTCTGCATTCTCGTTCCT	CCAGGACTCGTTGTACCCGTTG	NM_053275.3

<sup>#</sup>The IGF-IEc specific 49 base-pair insertion (Hameed *et al.* 2002) is discriminated by an insertion-specific sense primer (IGF-IEc) versus an insertion point overlapping primer (last two 3' bases) sense primer (IGF-IEa).

### Muscle and tendon mRNA measurements

The amount of mRNA for collagen  $\alpha 1(I)$  (COL1A1), collagen  $\alpha 1(III)$  (COL3A1), IGF-IEa and IGF-IEc was measured with real-time reverse transcriptase (RT) PCR (Table 1). Tendon and muscle were homogenized in TriReagent (Molecular Research Centre, Cincinnati, OH, USA) using a bead-mixer with steel beads (Biospec Products, Bartlesville, OK, USA). Following homogenization, bromo-chloropropane (Molecular Research Centre) was added in order to separate the samples into an aqueous and an organic phase. Glycogen was added to the tendon samples to improve RNA precipitation. Following isolation of the aqueous phase, RNA was precipitated using isopropanol, washed in ethanol and dissolved in RNase-free water. All tissue samples were weighed prior to RNA extraction. Muscle RNA concentrations were determined by spectroscopy and tendon RNA concentrations were determined using RiboGreen assay (Molecular Probes, Eugene, OR, USA). Good RNA quality was ensured by gel electrophoresis, which was combined with Northern blotting with radioactive probes for 28S and 18S ribosomal RNA for the smaller tendon samples.

Synthesis of complementary DNA (cDNA) was performed using the Omniscript reverse transcriptase (Qiagen, Hilden, Germany) on 500 ng of muscle RNA and Sensiscript reverse transcriptase (Qiagen) on 30 ng of tendon RNA, both in 20  $\mu$ l. For each target mRNA, 0.25  $\mu$ l cDNA was amplified in 25  $\mu$ l Quantitect SYBR Green Master Mix (Qiagen) with specific primers (100 nm each, Table 1) on a real-time PCR machine (MX3000P, Stratagene, La Jolla, CA, USA). The thermal profile was 95°C, 10 min  $\rightarrow$  (95°C, 15 s  $\rightarrow$  58°C, 30 s  $\rightarrow$  63°C, 90 s)  $\times$  50  $\rightarrow$  95°C, 60 s  $\rightarrow$  55°C, 30 s  $\rightarrow$  95°C, 60 s. Signal intensity was acquired at the 63°C step and the threshold cycle ( $C_t$ ) values were related to a standard curve made with the cloned PCR product. Specificity was confirmed by melting curve analysis after amplification (the 55°C to 95°C step). We analysed the tissue samples for both IGF-IEa and IGF-IEc. A 49 base-pair insertion in IGF-IEc makes this isoform distinguishable from IGF-IEa. In the IGF-IEa assay the sense primer was placed over

the insertion point ensuring that the last two bases fitted downstream of the insertion point. This creates a single base mismatch between IGF-IEa and IGF-IEc in the 3' end (. . .GGA-3' in IGF-IEa versus . . .GTA-3' in IGF-IEc), which should inhibit amplification from IGF-IEc cDNA (Wu *et al.* 2009). Indeed, there was no amplification of the 49 base-pair larger IGF-IEc isoform in the IGF-IEa assay, based on agarose gel and melting curve analysis. For the IGF-IEc assay, the sense primer was placed in the IGF-IEc specific insertion. The large ribosomal protein P0 (RPLP0) mRNA, which was stably expressed relative both to GAPDH mRNA and total RNA (data not shown), was chosen as internal control.

### Collagen and myofibrillar protein fractional synthesis rate (FSR)

Measurement of collagen and myofibrillar protein FSR was carried out according to previous techniques (Babraj *et al.* 2002). Plasma proline was used to establish the natural abundance of [<sup>15</sup>N] proline and [1-<sup>13</sup>C] proline, thereby eliminating the need for a pre-infusion basal tissue biopsy. To label collagen protein in muscle and tendon, a flooding dose of [<sup>15</sup>N] proline or [1-<sup>13</sup>C] proline (0.75–1 g labelled proline (>99 atoms%, Cambridge Isotope Laboratory, Andover, MA, USA) plus 3–3.25 g unlabelled proline (AppliChem, Darmstadt, Germany), 4 g total) was given. Blood samples were drawn every 10–30 min during the 2 h experiment to establish area under the curve (AUC) for the precursor. For extraction of proteins, tendon tissue was ground in liquid nitrogen to a fine powder, re-suspended in extraction buffer (0.02 M Tris-HCl, 0.15 M NaCl, 0.1 M EDTA, 0.1% Triton X-100, pH 7.4), and centrifuged (1600 g, 4°C) to pellet the collagen. The pellets were washed twice with 70% ethanol. Muscle tissue was ground in liquid nitrogen and re-suspended in extraction buffer. The homogenate was centrifuged (1600 g, 4°C), the supernatant removed and the myofibrillar/collagen pellet re-suspended in 0.3 M NaOH at 37°C for 20 min. The soluble myofibrillar protein and the insoluble collagen were separated by centrifugation. The myofibrillar fraction was precipitated using 1 M

perchloric acid and the pellet washed twice with 70% ethanol. For protein hydrolysis and gas-chromatography mass-spectrometry, protein from all sources was hydrolysed in 6 M HCl at 110°C overnight and the amino- and imino-acids purified using cation exchanger (Dowex 50WX8, Biorad, Copenhagen, Denmark) and eluted by 2 M NH<sub>4</sub>OH. The amino- and imino-acids were derivatized as their *N*-acetyl-*N*-propyl (NAP) esters. NAP amino- and imino-acids were analysed by capillary gas-chromatography–combustion–isotope-ratio mass-spectrometer (GC-C-IRMS) (Delta-plus XL, Thermo Finnigan, Bremen, Germany); separation was achieved on a 30 m × 0.25 mm, 1.5 μm film DB 1701 capillary column (J&W Scientific, Agilent Technologies, USA). Plasma proline enrichments were analysed as their *t*-butyldimethylsilyl derivative by gas-chromatography mass-spectrometry (Trace DSQ, Thermo Fisher Scientific, Hemel Hempstead, UK) using a EC-1, 30 m × 0.25 mm, 0.25 μm column (Econocap, Grace, Deerfield, USA). The rate of tissue protein synthesis was calculated according to the precursor-product principle as: Fractional protein synthesis (FSR% h<sup>-1</sup>) = ΔE<sub>p</sub> × 100/AUC<sub>p</sub>. ΔE<sub>p</sub> is the change in proline labelling over time in tissue protein and AUC<sub>p</sub> is the area under the curve of venous proline labelling with time in hours.

### Hormone assays

Blood samples drawn from the antecubital vein were separated (3200 g, 4°C) and serum was stored at -80°C. Serum GH (sGH) and serum IGF-I (sIGF-I) concentrations were determined by a time-resolved immuno-fluorometric assay (TR-IFMA, Perkin Elmer, Turku, Finland). Serum IGFBP-1 (sIGFBP-1) was determined by an in-house radioimmunoassay as described previously (Westwood *et al.* 1994). Serum IGFBP-3 (sIGFBP-3) was measured by commercially available immunoradiometric assay (IRMA, BioSource Europe, Nivelles, Belgium). The hormone assays have intra- and inter-assay coefficients of variation below 5 and 10%, respectively.

### Statistics

Using SAS (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA) the mixed linear model procedure was conducted for the effect of factors and interaction on tissue mRNA expression and protein FSR. Factors were rhGH/placebo, exercise/rest and period 1/period 2. Outcome was difference between groups with confidence interval (CI) and significance level. Subgroup/*post hoc* analysis was not conducted. mRNA data were log-transformed before statistical analysis and are presented as geometric mean ± back-transformed S.E.M.

Differences were considered significant when  $P < 0.05$ . For serum hormones, differences between rhGH *vs.* placebo were tested with paired *t* tests at the individual time points. Pearson's correlation analysis was used to determine baseline correlation between systemic IGF-I/local IGF-I mRNA and collagen mRNA. Subject characteristics are presented as mean ± S.D. All other results are presented as mean ± S.E.M.

### Results

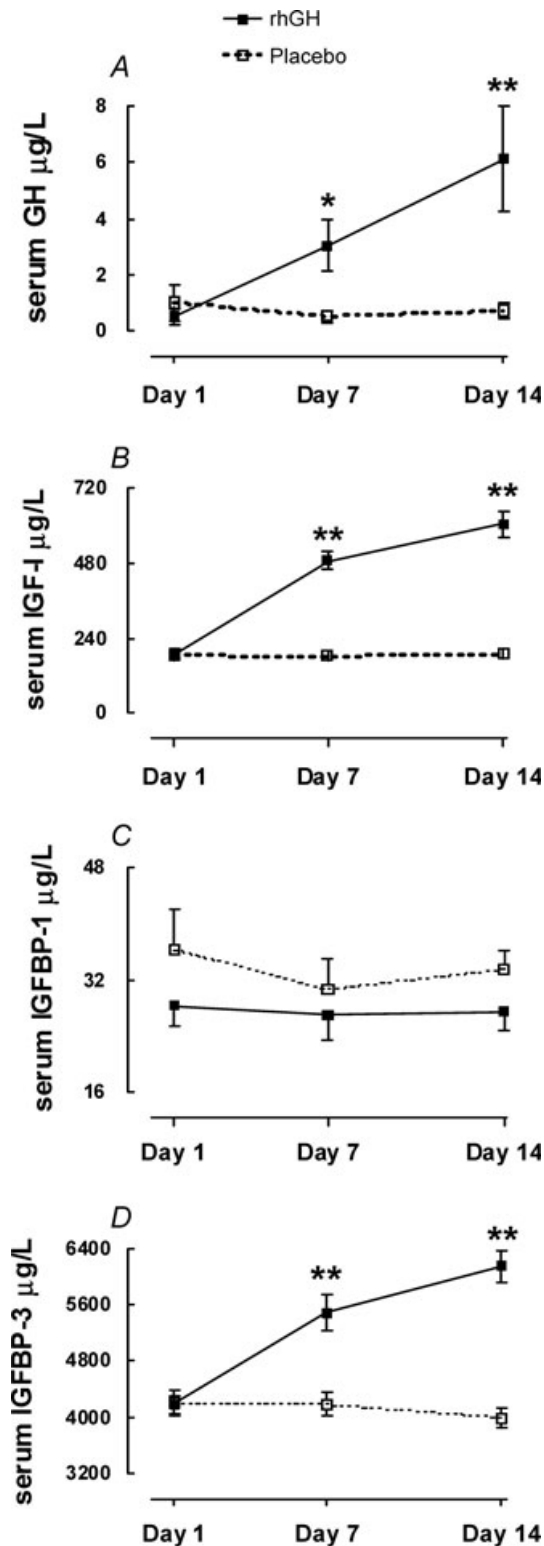
All 10 participants completed both rhGH and placebo trial periods, and were fully compliant with regard to rhGH/placebo administration according to GH-administration diaries and sGH and sIGF-I measurements.

#### Systemic GH and IGF-I

sGH was increased by 5.8-fold (95% CI: 1.3; 10;  $P = 0.02$ ) on day 7 and by 8.5-fold (95% CI: 3.0; 14;  $P = 0.01$ ) on day 14 of rhGH supplementation (Fig. 1A). sIGF-I was increased by 2.7-fold (95% CI: 2.3; 3.1;  $P < 0.01$ ) on day 7 and by 3.2-fold (95% CI: 2.7; 3.7;  $P < 0.01$ ) on day 14 of rhGH supplementation (Fig. 1B). sIGFBP-1 did not respond to rhGH supplementation (Fig. 1C), whereas sIGFBP-3 increased by 1.3-fold (95% CI: 1.1; 1.5;  $P < 0.01$ ) on day 7 and by 1.5-fold (95% CI: 1.4; 1.7;  $P < 0.01$ ) on day 14 of rhGH supplementation (Fig. 1D).

#### Design

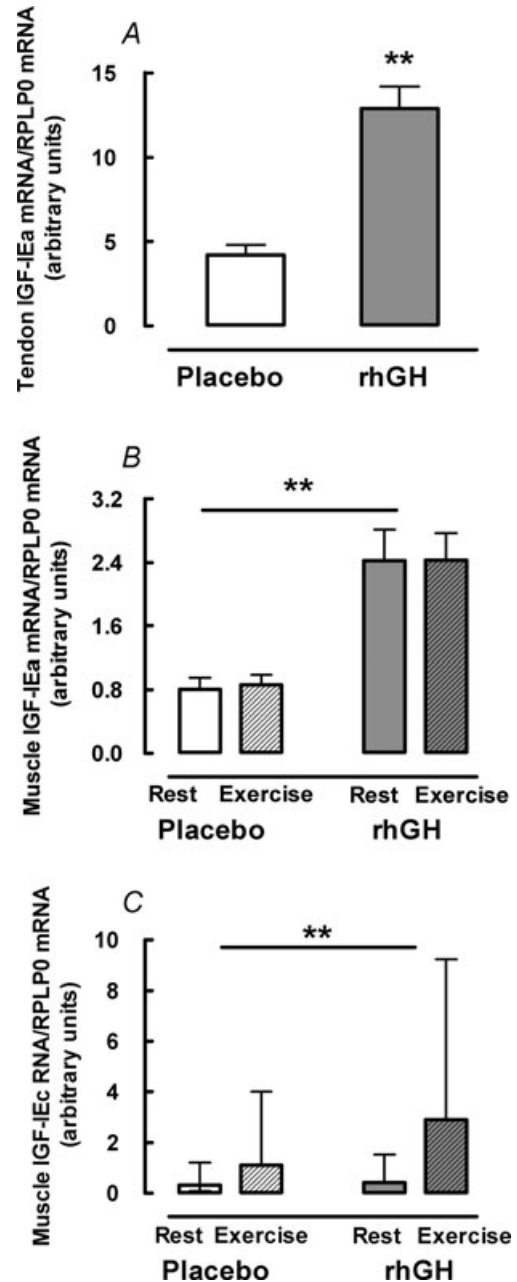
The crossover design necessitated two biopsies from each patella tendon. In order to avoid a carry-over effect from the first to the second tendon biopsy, the two biopsies were separated by 5 months and the anatomical locations of the biopsies were separated by at least 1 cm. There was, however, a significant carry-over effect of the repeated biopsy (referred to as period-effect in the text) on the concentration of collagen I and III mRNA in tendon (both  $P < 0.01$ ). Although interesting in itself, this required additional statistical evaluation. The period-effect interacted significantly with exercise for both collagen I and III mRNA ( $P < 0.01$ ), making interpretation of the individual effect of period and exercise on tendon collagen mRNA complicated. There was no period-effect on tendon IGF-IEa mRNA ( $P = 0.26$ ) but period-effect and exercise interacted significantly for tendon IGF-IEa mRNA ( $P = 0.01$ ). Most importantly, there was no interaction between rhGH supplementation and period-effect ( $P > 0.14$ ) or rhGH supplementation and exercise ( $P > 0.81$ ) for either collagen I, collagen III or IGF-IEa mRNA. This shows that the effect of rhGH treatment on mRNA for collagen I, III and IGF-IEa in



**Figure 1. Circulating concentrations of GH, IGF-I, IGF-BP-1 and IGF-BP-3 in young male participants ( $n = 10$ ) that were studied over 14 days in a crossover design with rhGH/placebo supplementation**

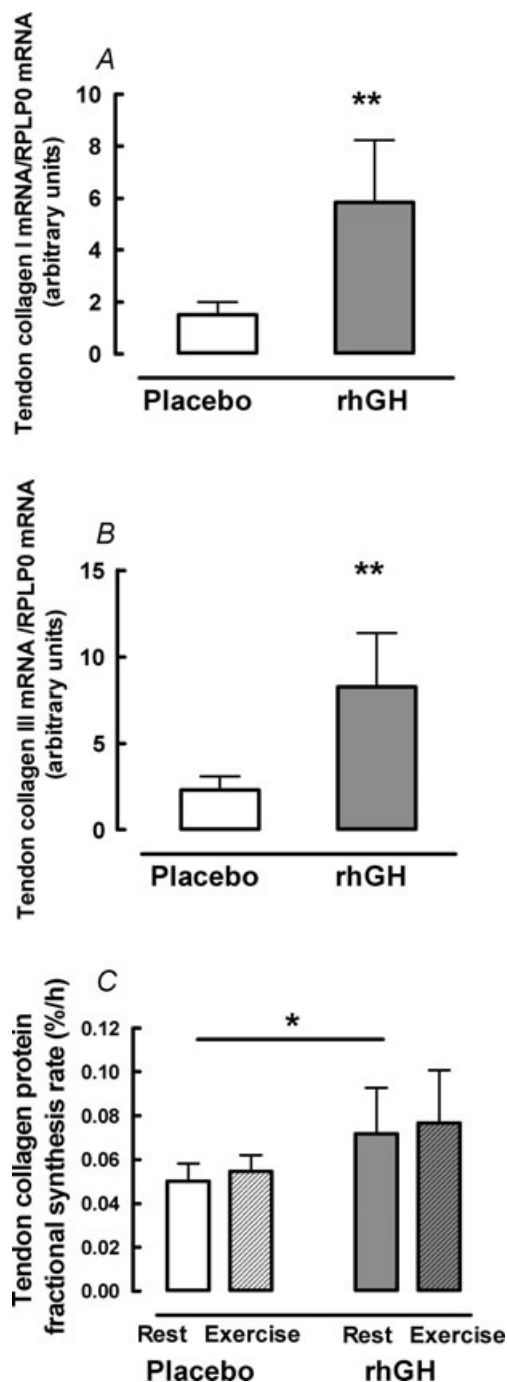
Data are means  $\pm$  s.e.m. \*Difference between rhGH supplementation and placebo  $P < 0.05$ . \*\*Difference between rhGH and placebo  $P < 0.01$ .

tendon was not influenced by the level of period-effect or by exercise. Data for tendon collagen I, III and IGF-IEa mRNA expression are presented as differences between placebo and rhGH supplementation only (Figs 2A, and 3A and B). Essentially, there was no period-effect on tendon collagen protein FSR ( $P = 0.45$ ) or on any of the measured



**Figure 2. Local IGF-I isoform mRNA expression in human skeletal muscle and tendon**

Young male participants ( $n = 10$ ) were studied over 14 days in a crossover design with rhGH/placebo supplementation. Data for exercise effect on tendon mRNA is not shown due to repeated biopsy effect (see text for details). Data are geometric means  $\pm$  back-transformed s.e.m. A, tendon IGF-IEa. B, muscle IGF-IEa. C, muscle IGF-IEc. \*\*Difference between rhGH and placebo  $P < 0.01$ .



**Figure 3. Tendon collagen mRNA expression and protein fractional synthetic rate**

Young male participants ( $n = 10$ ) were studied over 14 days in a crossover design with rhGH/placebo supplementation. Data for exercise effect on tendon mRNA are not shown due to repeated biopsy effect (see text for details). Data are geometric means  $\pm$  back-transformed s.e.m. *A*, collagen I mRNA. *B*, collagen III mRNA. *C*, protein fractional synthesis rate. \*Difference between rhGH and placebo  $P < 0.05$ . \*\*Difference between rhGH and placebo  $P < 0.01$ .

variables in skeletal muscle ( $P > 0.25$ ), where biopsies were taken more than 5 cm apart.

### Tendon

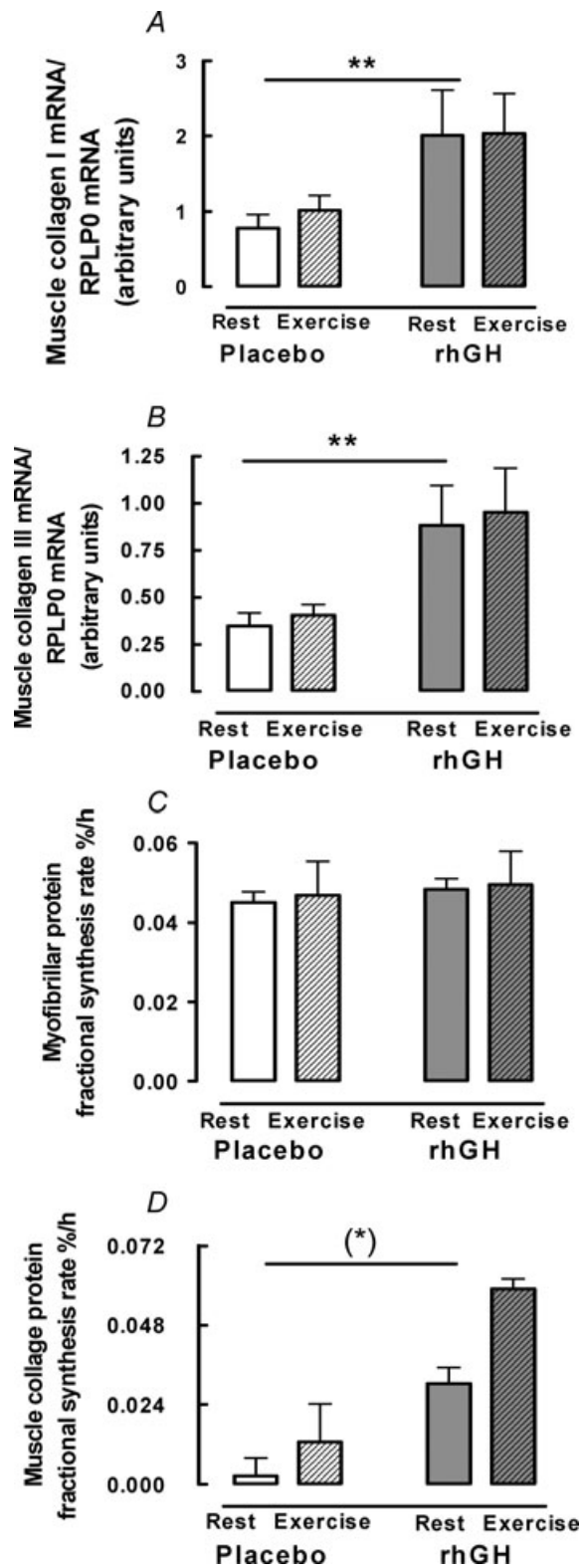
Tendon IGF-IEa mRNA increased 3.0-fold (95% CI: 2.3; 3.9;  $P < 0.01$ ) after rhGH supplementation (Fig. 2*A*). IGF-IEc mRNA concentration was below the detection limit in 30% of the tendon biopsies and in the remaining samples there was no effect of rhGH ( $P = 0.31$ ) or exercise ( $P = 0.91$ ) (not shown). rhGH supplementation increased collagen I mRNA by 3.9-fold (95% CI: 2.0; 7.8;  $P < 0.01$ ) and collagen III mRNA by 3.6-fold (95% CI: 2.0; 6.4;  $P < 0.01$ ) (Fig. 3*A* and *B*). Tendon collagen protein FSR was 1.3-fold (95% CI: 1.1; 1.5;  $P = 0.02$ ) higher after rhGH supplementation than placebo (Fig. 3*C*). Prior exercise did not affect collagen mRNA (data not shown due to repeated biopsy effect) or collagen protein FSR ( $P > 0.44$ ) and there was no interaction between rhGH and exercise ( $P = 0.95$ ) either for collagen mRNA or protein synthesis (Fig. 3).

### Muscle

In muscle, IGF-IEa mRNA was 2.9-fold higher (95% CI: 2.1; 3.9;  $P < 0.01$ ) and IGF-IEc mRNA was 5.0-fold higher (95% CI: 3.3; 7.5;  $P < 0.01$ ) with rhGH supplementation relative to placebo (Fig. 2*B* and *C*). There was no effect of exercise on IGF-IEa ( $P = 0.72$ ) or IGF-IEc ( $P = 0.23$ ) in muscle and no interaction between rhGH and exercise on either isoform ( $P > 0.74$ ) (Fig. 2*B* and *C*). Relative to placebo, rhGH supplementation increased mRNA for collagen I by 2.3-fold (95% CI: 1.3; 4.0;  $P < 0.01$ ) and collagen III by 2.5-fold (95% CI: 1.5; 3.9;  $P < 0.01$ ) (Fig. 4*A* and *B*). Although not statistically significant, a tendency towards a 5.8-fold (95% CI: 0.9; 11.8;  $P = 0.06$ ) increase in muscle collagen protein FSR was observed following rhGH supplementation (Fig. 4*D*). Exercise did not affect collagen I mRNA ( $P = 0.44$ ), collagen III mRNA ( $P = 0.47$ ) or collagen protein FSR ( $P > 0.10$ ) and there was no interaction between rhGH supplementation and exercise for any collagen mRNA or collagen protein FSR ( $P > 0.49$ ) (Fig. 4). Muscle myofibrillar protein FSR was not affected by rhGH ( $P = 0.75$ ) or exercise ( $P = 0.10$ ) and there was no interaction between rhGH and exercise ( $P = 0.49$ ) (Fig. 4*C*).

### IGF-I-collagen correlation

Local tissue IGF-I mRNA expression correlated significantly with collagen I mRNA expression in both tendon ( $r^2 = 0.48$ ,  $P = 0.03$ ) and muscle ( $r^2 = 0.33$ ,  $P = 0.02$ ) (not shown). Serum IGF-I did not correlate significantly with collagen I mRNA expression in



**Figure 4. Muscle collagen mRNA expression and protein fractional synthesis rate, and muscle myofibrillar protein fractional synthesis rate**

Young male participants ( $n = 10$ ) were studied over 14 days in a crossover design with rhGH/placebo supplementation. Data are mean  $\pm$  S.E.M. *A*, collagen I mRNA. *B*, collagen III mRNA.

either tendon ( $r^2 = 0.34$ ,  $P = 0.08$ ) or muscle ( $r^2 = 0.11$ ,  $P = 0.20$ ) (not shown).

## Discussion

The major finding in the present study is that increased GH availability causes increased expression of collagen mRNA and also increased collagen protein synthesis in connective tissue in human skeletal muscle and tendon, but has no effect on myofibrillar protein synthesis. Moreover, it was demonstrated that serum IGF-I, as well as tissue IGF-I mRNA expression was related to serum GH titre. These findings demonstrate that GH caused a rise in matrix collagen synthesis of skeletal muscle and tendon, but did not reveal any effect on myofibrillar protein synthesis.

### Effect of GH on systemic and local IGF-I

As expected, sIGF-I and sIGFBP-3 availability was related to sGH titre. Supplementation with rhGH, which was gradually increased over time, led to a gradual increase in sIGF-I/IGFBP-3 following the pattern of the sGH increase (Fig. 1). This observation is of fundamental importance for the interpretation of the results as a whole, because it confirmed that the rhGH supplementation was sufficient to stimulate the GH/IGF-I axis.

An important finding was that local IGF-I mRNA expression increased in both tendon and muscle in response to rhGH supplementation. The finding that elevating GH not only increases IGF-I systemically but also results in an elevated expression of IGF-IEa mRNA locally in tendon tissue in man is, to our knowledge, novel and supports the observation of GH-induced rise in mRNA for both IGF-I and collagen in skeletal muscle of hypopituitary men (Sjogren *et al.* 2007). It is also consonant with the described effect of GH in several animal tissues (Butler & Le Roith, 2001) and it is supported by earlier observations made in human skeletal muscle after 5 and 12 weeks of rhGH supplementation in elderly subjects (Hameed *et al.* 2004). The finding that IGF-IEc is present in human tendon, albeit in small amounts, is novel and is supported by observations in rats (Heinemeier *et al.* 2007b).

### GH/IGF-I effect on connective tissue

A primary observation was that the GH/IGF-I axis appeared to be closely involved in regulation of

*C*, myofibrillar protein fractional synthesis rate. *D*, collagen protein fractional synthesis rate. (\*) Difference between rhGH and placebo  $P = 0.06$ . \*\*Difference between rhGH and placebo  $P < 0.01$ .

the connective tissue supporting skeletal muscle. Administration of rhGH increased collagen I and III mRNA 2-fold in both tendon and muscle, and collagen protein FSR 1.3-fold and 6-fold in tendon and muscle, respectively (Figs 3 and 4).

Reports showing a 2-fold increase in the concentration of markers for collagen synthesis (pro-collagen III pro-peptide (PIIIP)) in human adults following GH supplementation (Longobardi *et al.* 2000; Powrie *et al.* 2007) and appropriately altered PIIIP concentrations in acromegalic and growth hormone-deficient (GHD) patients (Ueland *et al.* 2006; Gonc & Kandemir, 2007) are compatible with our observations of increased collagen mRNA expression and synthesis after GH elevation. These studies support the view that GH stimulates collagen synthesis; nevertheless, as serum PIIIP only indirectly reflects whole body collagen synthesis, such observations are clearly inconclusive regarding changes in the collagen matrixes in muscle and tendon. Other results from animal work support our conclusions (Wilson *et al.* 1995), although intramuscular GH injections did not affect tendon collagen synthesis in pigs until after 3 months (Choy *et al.* 2005).

### Importance of IGF-I signalling

The observed effect of rhGH supplementation on collagen synthesis was possibly mediated by IGF-I signalling. A significant correlation between local IGF-I mRNA and collagen mRNA in both muscle and tendon was demonstrated, whereas no significant correlations between sGH or sIGF-I and collagen expression were observed in the rhGH-supplemented participants. This suggests a role for local IGF-I signalling, a notion that is supported by animal studies, showing that circulating IGF-I has little effect on overall growth in mice (Sjogren *et al.* 1999), while locally produced IGF-I is a prerequisite for GH stimulatory effects in muscle and cartilage (Schlechter *et al.* 1986; Kim *et al.* 2005). IGF-I is known to induce collagen synthesis in animal tendon explants (Abrahamsson *et al.* 1991; Banes *et al.* 1995; Murphy & Nixon, 1997), and in mice that over-express human IGF-I locally it was demonstrated that the collagen content was elevated in heart muscle (Delaughter *et al.* 1999). These findings are in accordance with our results and support the view that IGF-I rather than GH directly is responsible for the elevated collagen expression and protein synthesis seen in the present study (Figs 3 and 4).

### Muscle fibrillar protein synthesis

In contrast to the effect on connective tissue, elevated GH did not increase myofibrillar protein synthesis. With rhGH supplementation, both circulating and local IGF-I was

increased by 3-fold but this did not enhance myofibrillar protein FSR (Fig. 4). This finding is in contrast to the well-established positive effect of GH and IGF-I on muscle protein and strength found in animals with a large growth potential (Musaro *et al.* 2001; Quinn *et al.* 2007), but it is in concert with previous human experiments where no effect on muscle strength and protein FSR was observed (Yarasheski *et al.* 1993; Blackman *et al.* 2002; Lange *et al.* 2002; Berggren *et al.* 2005; Ehrnborg *et al.* 2005). So rather than causing muscle fibre growth, GH/IGF-I appear to stimulate the supporting connective tissue that would help force transmission from the contracting muscle fibres to the bone.

### Exercise effect

Based on previous observations of a positive correlation between mechanical loading and collagen synthesis in human muscle and tendon (Miller *et al.* 2005; de Boer *et al.* 2007), we attempted to introduce a local exercise-induced stimulation of protein synthesis. This was done to study a possible interaction between the effects of exercise and IGF-I status, as suggested *in vitro* (Banes *et al.* 1995). Although the positive effect of GH on local collagen production persisted during exercise, with rhGH supplementation there was somewhat surprisingly no effect of exercise *per se* on collagen or myofibrillar protein synthesis and no interactions between GH and exercise. The explanation probably lies in the exercise mode. In our study we aimed at giving a well-defined bout of concentric resistance exercise with high load with a known potential to increase mixed muscle protein synthesis (Phillips *et al.* 1997; de Boer *et al.* 2007). However, the total number of repetitions was only 100, in contrast to approximately 2000 light load repetitions in the study by Miller *et al.* (2005), and may therefore have been insufficient to create a robust exercise-induced response on collagen synthesis. In this context, our findings suggest that the total number of repetitions, rather than the load, is important in eliciting a response in collagen and myofibrillar protein synthesis measured 24 h post exercise.

### Clinical perspectives

In this study, just 14 days of rhGH supplementation in healthy individuals increased collagen synthesis by up to 6-fold without causing any side effects. An increase of this magnitude holds clinical perspectives in relation to traumatic musculoskeletal injuries, where the collagen matrix inevitably is damaged (James *et al.* 2008). In animals, local and systemic application of IGF-I has been shown to increase both tendon collagen content and force-to-failure in relation to both overuse and acute injuries (Dahlgren *et al.* 2002; Provenzano *et al.* 2007),



and similar observations are made in animal skin and bone (Dunaiski & Belford, 2002; Andreassen & Oxlund, 2003). Very few studies have investigated the effects of GH or IGF-I treatment in relation to human musculoskeletal tissue injuries. Tentatively indicative of a beneficial effect of GH treatment is a case control study with six patients, in whom application of a mixture of growth factors, including IGF-I, increased foot range of motion and decreased recovery time after Achilles tendon rupture (Sanchez *et al.* 2007). However, more convincing evidence is given in a placebo-controlled trial with 406 patients, in whom a significantly shorter time to healing of closed fractures was observed after high-dose GH treatment (Raschke *et al.* 2007). This is consonant with the increased collagen synthesis in muscle and tendon we observed after GH treatment given in similar high doses.

## Conclusion

In conclusion, we found that rhGH administration caused a rise in matrix collagen synthesis in skeletal muscle and tendon, but had no effect on myofibrillar protein synthesis. The observed increase in both systemic IGF-I and tissue IGF-I mRNA suggests IGF-I signalling. Thus, GH/IGF-I apparently reinforces the supporting collagen framework around muscle fibres rather than the muscle contractile apparatus *per se* in adult skeletal muscle. GH/IGF-I may be more biologically important for strengthening the supportive matrix in tissues than for muscle cell hypertrophy in adult human musculotendinous tissue.

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The experiments were performed at the Institute of Sports Medicine, Bispebjerg Hospital, Center of Healthy Aging, Faculty

of Health Sciences, University of Copenhagen, Bispebjerg Bakke 23, DK-2400 Copenhagen NV, Denmark. All the authors have contributed to conception, design, analysis, interpretation of data, drafting and revising the manuscript, as well as to the final approval of the version to be published.

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