Insulin-like growth factor-I, actin, and myosin heavy chain messenger RNAs in skeletal muscle after an injection of growth hormone in subjects over 60 years old

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Abstract

Growth hormone (GH) increases the amount of insulin-like growth factor-I (IGF-I) mRNA in rat skeletal muscle, but this effect has not been demonstrated in human muscle. An autocrine effect of IGF-I produced in muscle may be an important determinant of the increased muscle mass associated with GH therapy. Thus, we examined IGF-I mRNA abundance in skeletal muscle biopsy samples taken 10 h after a subcutaneous injection of GH (0·03 mg/kg, n=6) or placebo (normal saline, n=5) in men and women over 60 years of age. Relative tissue concentrations of IGF-I mRNA were evaluated with a competitive reverse transcriptase-polymerase chain reaction assay. Mean plasma IGF-I concentrations rose steadily after the GH injection, and were 74% higher in the GH group than in the control group at the time of the muscle biopsies. There was no consistent difference between the GH and control groups in muscle IGF-I mRNA abundance when expressed in relation to total RNA or polyadenylated RNA. However, one GH-treated subject had three times more IGF-I mRNA, relative to polyadenylated RNA, than the average control subject. There was no effect of GH on levels of mRNAs encoding the most abundant myofibrillar proteins, actin and myosin heavy chain. These data do not support the hypothesis that increased IGF-I mRNA abundance in skeletal muscle is required for the anabolic effect of GH in people over 60 years of age.

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Introduction

The stimulation of insulin-like growth factor-I (IGF-I) production by growth hormone (GH) is mediated by increased transcription of the IGF-I gene, leading to increased abundance of IGF-I mRNA (Adamo 1995). This effect of GH has been observed in several tissues of rats, including skeletal muscle (Murphy et al. 1987, Turner et al. 1988, Isgaard et al. 1989, Gosteli-Peter et al. 1994). However, GH stimulation of IGF-I mRNA expression has not been demonstrated in human skeletal muscle. The pattern of tissue IGF-I production could be an important determinant of the effectiveness of GH as a promoter of lean tissue growth or preservation in old age. Taaffe et al. (1996) recently reported that abundance of IGF-I mRNA in skeletal muscle of healthy 65- to 82-year-old men did not increase after 10 weeks of daily GH administration (0·02 mg/kg, s.c.). Although they obtained muscle within 24 h of the final GH injection, it is likely that circulating GH levels had declined to normal levels several hours before the time of biopsy. Moreover, it is possible that GH elicited an increase in muscle IGF-I mRNA earlier in the course of GH treatment. We have examined expression of IGF-I mRNA in skeletal muscle while plasma GH levels were elevated after an injection of GH in healthy older subjects. We also examined the effect of GH on expression of the mRNAs encoding the abundant myofibrillar proteins actin and myosin heavy chain, because it has been reported that myosin heavy chain mRNA increases after a GH infusion in young men (Fong et al. 1989).

Materials and Methods

The subjects were 8 men and 3 women, 64–74 years old. All were healthy according to medical history, physical examination, and laboratory tests (glucose tolerance test, serum electrolytes, albumin, creatinine, liver enzymes, thyrotropin, blood count, blood clotting profile), were non-smokers, and were not taking any prescription medications. All subjects gave informed, written consent to participate after procedures were explained verbally and in writing. The protocol was approved by the University of Rochester Research Subjects Review Board.
Muscle biopsy samples for the present analyses were obtained during a study of the effect of GH on muscle protein synthesis, as reported in a separate paper (Welle et al. 1996b). That aspect of the study required a tracer infusion, serial blood sampling, and an earlier muscle biopsy from the contralateral leg, but did not involve any procedures that might influence the effect of GH on IGF-I mRNA expression. A sample of the vastus lateralis was obtained within a few minutes after a local anesthetic was injected in the muscle, 10 h after administration of either recombinant human GH (0.03 mg/kg, s.c., Genentech, South San Francisco, CA, USA) or placebo (normal saline). Blood samples were obtained for plasma GH and IGF-I concentrations at 2-h intervals after the injection, which was given at approximately 0530 h. Six subjects (four men, two women) received GH and five (four men, one woman) received placebo.

Tissue RNA extraction and determination of total RNA, polyadenylated RNA, actin mRNA, and myosin heavy chain mRNA were carried out as described previously (Welle et al. 1996a). IGF-I mRNA was determined with a competitive reverse transcriptase-polymerase chain reaction (RT-PCR) assay. Primers (A: GCTGGTG GATGCTCTTCCGTT; B: AGCTGACTTGGCCAG GCTTGGAG) were chosen to amplify a 184 base segment of the coding region of human IGF-I mRNA (Rotwein et al. 1986, Steenbergh et al. 1991). Identity of the PCR product was verified by sequencing. The segment contained sequences from widely separated exons (2 and 3) of the IGF-I gene, so that amplification of any residual DNA present in the RNA preparation could not interfere with the assay. The internal standard was a 159 base RNA that was identical to the amplified segment of the IGF-I mRNA except for an internal deletion of 25 bases. The deletion was accomplished by extending primer A with the sequence TTTCAACGCCACAGGATGGTG, and using it to amplify the product (cut from an agarose gel) obtained from RT-PCR (using primers A and B) of human muscle RNA. The T7 polymerase promoter was added to the resulting product by amplifying it with a primer containing the promoter sequence (TAATACG ACTCAGTATAGGGAG) at the 5’ end of primer A. The resulting DNA was transcribed with T7 polymerase to obtain the standard RNA.

Random hexamers were used to prime an RT reaction with 0.5 µg total RNA from the muscle sample and 1 pg standard RNA. The resulting cDNAs were amplified with primers A and B with the following PCR parameters: hot start at 94 °C for 30 s, 32 cycles with temperature held at 62 °C for 30 s and at 94 °C for 30 s, plus a final cycle with 6 min at 72 °C. Use of the standard RNA in the RT-PCR assay controls for variations between samples in RT and polymerase enzyme efficiency. The PCR products were separated on a polyacrylamide gel, stained with SYBR Green (Molecular Probes, Eugene, OR, USA), and photographed through a yellow filter during exposure to UV light. Band intensity was assessed from the photographs using the Microcomputer Imaging Device (Imaging Research, St Catharines, Ontario, Canada). The ratio of sample to standard band intensity was used as the index of relative IGF-I mRNA abundance. Because exactly the same priming sequences are present in the IGF-I mRNA and the standard, and the length of the PCR product is similar except for the small deletion in the standard, this ratio is constant even if PCR cycle number exceeds the exponential phase of amplification. The assay was performed in duplicate, and the mean value was used for data analysis. IGF-I mRNA abundance is expressed in arbitrary units, with a value of 1·0 being the mean of the placebo group. The mean deviation due to assay variability was 0·2 arbitrary units (maximum deviation=0·4 arbitrary units).

Commercial immunoradiometric assay kits were used to measure plasma concentrations of GH (Nichols Institute, San Juan Capistrano, CA, USA) and IGF-I (Diagnostic Systems Laboratories, Webster, TX, USA).

Data are expressed as the mean ± one standard error. The statistical significance of differences between the means of the GH and placebo groups was determined by t-test.

Results

Mean plasma GH levels did not exceed 2.2 ng/ml after the placebo injection. GH concentrations were significantly elevated for the entire 10-h period that they were measured after the GH injection, peaking at 15·4 ± 3·6 ng/ml at 4 h before gradually declining (Fig. 1). Plasma IGF-I concentrations did not change after the placebo injection. They increased approximately 100% (P<0.001) between 2 and 10 h after the GH injection, and were 74% higher (P<0.02) in the GH group than they were in the placebo group at the time of the muscle biopsy (Fig. 1).

There was no significant difference in the amount of total RNA recovered from the muscle samples in the placebo (0·32 ± 0·05 µg/mg tissue) and the GH groups (0·36 ± 0·03 µg/mg tissue). Equal amounts of total RNA from each sample were analyzed in slot blot assays for polyadenylated RNA, actin mRNA, and myosin heavy chain mRNA. The results of these assays are presented in Table 1, with data expressed relative to the mean value in the placebo group. The RNA from the GH group had slightly less polyadenylated RNA than the RNA from the placebo group, but the difference was not statistically significant. Actin mRNA abundance was slightly less (P<0.05) in the GH group relative to total RNA, but not relative to polyadenylated RNA. Myosin heavy chain mRNA was not significantly different in GH and placebo.
groups, whether expressed in relation to total RNA or polyadenylated RNA.

Equal amounts of total RNA were analyzed in the competitive RT-PCR assays for IGF-I mRNA. There was no significant difference ($P > 0.50$) in the mean abundance of IGF-I mRNA per µg total RNA between the GH group and the placebo group (Table 2). The mean ratio of IGF-I mRNA to polyadenylated RNA was 50% higher in the GH group (Table 2), but the difference was not statistically significant ($P > 0.20$). Most of this difference was caused by a single GH-treated subject whose IGF-I mRNA abundance, relative to polyadenylated RNA, was threefold greater than the mean abundance in the placebo group (Table 2). This subject had the highest peak plasma GH and IGF-I concentrations (Table 2).

### Discussion

The present results agree with the recent report of Taaffe et al. (1996), who found no consistent increase in IGF-I mRNA in muscles of healthy old men after 10 weeks of GH treatment. In that study, the mean IGF-I mRNA level in muscle, relative to ribosomal RNA, was 55% higher after GH treatment than it was before treatment. Taaffe et al. (1996) noted that this effect was attributable to a 'substantial' response in 2 of 8 subjects, with no effect of GH on IGF-I mRNA in the other 6 subjects. We observed a 50% increase in the mean muscle IGF-I mRNA abundance relative to polyadenylated RNA (only 10% increase relative to total RNA) in GH-treated subjects. This effect was mainly attributable to one subject. Thus, while subjects over 60 years of age do not consistently have elevated levels of IGF-I mRNA in muscle after GH administration, a minority of them may have a significant response.

Because of assay variability (~20% of the mean control value), the relatively small number of subjects, and the unpaired study design (no pre-GH muscle available in GH-treated subjects), we cannot discount the possibility that there was a minor, undetected effect of GH on IGF-I mRNA in the muscles of most of the subjects. However, it is clear that GH does not usually induce several-fold increases in IGF-I mRNA in older human muscle, as has been observed in muscles of GH-treated hypophysectomized rats (Murphy et al. 1987, Isgaard et al. 1989, Gosteli-Peter et al. 1994) or muscles of rats implanted with GH-secreting cells (Turner et al. 1988).

Even though older subjects have a reduced GH secretion relative to young subjects, they still have significant nocturnal GH secretion, which might be adequate to allow full expression of the GH-dependent component of IGF-I gene transcription in the muscles of most subjects.

We cannot rule out the possibility that increased IGF-I mRNA occurred at earlier or later time points after the GH injection. However, the 10-h time point seemed optimal, based on GH-induced increases in IGF-I mRNA in rat muscle (Murphy et al. 1987, Isgaard et al. 1989) and porcine liver and adipose tissue (Ramsay et al. 1995), for finding such an effect. GH levels were still elevated at that point, had been elevated for several hours, and continued to stimulate production of IGF-I secreted into plasma (presumed to be mostly produced in the liver).

It is possible that a higher dose of GH would have consistently increased IGF-I mRNA in muscle. The only subject with evidence of a significant IGF-I mRNA response to GH in muscle had the highest peak GH level. If higher GH levels are needed to stimulate muscle IGF-I mRNA expression, the physiological relevance would be

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**Figure 1** Mean ± S.E.M. plasma concentrations of GH and IGF-I, 2–10 h after a subcutaneous injection of GH (solid lines, circles) or placebo (broken lines, squares). Muscle biopsy for muscle mRNA determinations was taken at 10 h.

**Table 1** Polyadenylated RNA, actin mRNA, and myosin heavy chain mRNA relative abundance in skeletal muscle 10 h after injection of GH or placebo. Results are the mean ± S.E., expressed as a proportion of the mean value in the placebo group

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>GH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyadenylated RNA/total RNA</td>
<td>1.0 ± 0.1</td>
<td>0.8 ± 0.03</td>
</tr>
<tr>
<td>Actin mRNA per total RNA</td>
<td>1.0 ± 0.1</td>
<td>0.7 ± 0.06*</td>
</tr>
<tr>
<td>Myosin heavy chain mRNA per total RNA</td>
<td>1.0 ± 0.04</td>
<td>0.9 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Polyadenylated RNA/total RNA</td>
<td>1.0 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

* $P < 0.05$ compared with placebo group.
questionable because normal episodes of GH secretion in humans do not usually result in GH levels higher than those achieved with the dose of GH given in the present study. Moreover, the dose used in the present study, when given 3 times per week, has been shown to increase lean body mass and muscle mass in men over 60 years old (Rudman et al. 1990, Welle et al. 1996). Thus, it seems unlikely that increased IGF-I mRNA in muscle is required for an anabolic effect of GH. Only one of the subjects whose data are presented in Table 2 (subject 2 in the GH group) received GH injections for 3 months in the longer trial previously described (Welle et al. 1996b). He had the typical increase in lean body mass and muscle mass, even though there is no evidence of elevated IGF-I mRNA after a GH injection in the present study.

Humans are not the only species whose IGF-I expression in muscle appears to be relatively insensitive to GH. Dwarf chickens, which lack GH receptors, do not express IGF-I mRNA in liver, but have a normal expression of IGF-I mRNA in muscle and other extrahepatic tissues (Tanaka et al. 1996). In pigs, an injection of porcine GH (0·2 mg/kg, 7 times more per kg body weight than the dose used in the present study) increased IGF-I mRNA in liver and adipose tissue, but did not increase it in latissimus dorsi or semitendinosus muscles, and increased it only slightly in the vastus lateralis (Ramsay et al. 1995). Daily GH injections in pigs (0·035–0·14 mg/kg for 7–24 days) increased IGF-I mRNA in liver and adipose tissue but not in muscle (Grant et al. 1991, Coleman et al. 1994).

Fong et al. (1989) reported that an intravenous infusion of GH for 6 h in young men increased abundance, relative to total tissue RNA, of myosin heavy chain mRNA. They suggested that increased abundance of the mRNAs encoding myofibrillar proteins could result in an increase in myofibrillar protein synthesis during GH treatment. We did not observe any difference in myosin heavy chain or actin mRNA abundance between GH– and placebo-treated subjects in the present study. Whereas stimulation of muscle protein synthesis by GH has been noted in young human subjects (Fryburg et al. 1991, Fryburg & Barrett 1993), GH did not increase myofibrillar protein synthesis in the present study (Welle et al. 1996b) and did not increase total muscle protein synthesis in older subjects in another study (Yarasheski et al. 1995). A direct comparison of young and old subjects in the same study is needed to establish whether the failure of GH to stimulate myofibrillar protein synthesis and increase abundance of the mRNAs encoding actin, myosin heavy chain, and IGF-I in older people can be explained by a diminished responsiveness to GH.

Acknowledgements

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Table 2 IGF-I mRNA relative abundance in skeletal muscle and plasma IGF-I concentrations 10 h after an injection of GH or placebo, and peak and mean plasma GH concentrations 2–10 h after an injection of GH or placebo. Values for mRNA levels are expressed as a proportion of the mean value in the placebo group.

<table>
<thead>
<tr>
<th>Subject number</th>
<th>Placebo group</th>
<th>GH group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per total RNA</td>
<td>Per poly(A) RNA</td>
</tr>
<tr>
<td>1</td>
<td>0·7</td>
<td>1·1</td>
</tr>
<tr>
<td>2</td>
<td>0·9</td>
<td>1·0</td>
</tr>
<tr>
<td>3</td>
<td>1·5</td>
<td>1·1</td>
</tr>
<tr>
<td>4</td>
<td>0·7</td>
<td>0·8</td>
</tr>
<tr>
<td>5</td>
<td>1·2</td>
<td>1·0</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>1·0 ± 0·15</td>
<td>1·0 ± 0·06</td>
</tr>
</tbody>
</table>

Poly(A) RNA, polyadenylated RNA.
*P < 0·02 compared with placebo group.
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