Muscle mechano growth factor is preferentially induced by growth hormone in growth hormone-deficient *lit/lit* mice

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Two muscle insulin-like growth factor-I (IGF-I) mRNA splice variants (IGF-IEa and IGF-IEb) have been identified in rodents. IGF-IEb, also called mechano growth factor (MGF) has been found to be upregulated by exercise or muscle damage. Growth hormone (GH) is the principal regulator of IGF-I expression in several tissues including skeletal muscle. Therefore, we investigated the effect of chronic GH excess or disruption of GH receptor (GHR) signalling, and the acute effect of GH administration on expression of muscle IGF-I isoforms using transgenic mice that express bovine GH (bGH), GHR gene-disrupted (GHR–/–) mice and GH-deficient *lit/lit* mice before and after exogenous GH administration. MGF mRNA in skeletal muscle was increased in bGH mice whereas it was decreased in GHR–/– mice compared with control animals. Exogenous GH administration to dwarf *lit/lit* mice significantly increased muscle MGF but not IGF-IEa mRNA 4 h after treatment. Twelve hours after GH treatment, both MGF and IGF-IEa mRNAs in muscle were increased compared with vehicle-treated *lit/lit* mice. In contrast in GH-sufficient *lit/+* mice, both MGF and IGF-IEa mRNAs were increased 4 h after and returned to the basal level 12 h after GH treatment. Hepatic IGF-I isoforms were regulated in parallel by GH. Thus, our results demonstrated that: (1) MGF mRNA in skeletal muscle is expressed in parallel with GH action; (2) MGF mRNA in muscle is produced preferentially in the situation of GH deficiency in contrast to the pattern in the GH-sufficient state; and (3) the induction of IGF-I isoforms by GH is tissue-specific.

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Insulin-like growth factor-I (IGF-I) is a ubiquitous peptide that stimulates skeletal growth and cell differentiation in an autocrine/paracrine as well as endocrine fashion (D'Ercole *et al.* 1984; Stewart & Rotwein, 1996). Previous work indicates that IGF-I is involved in the maintenance of skeletal muscle tissue and also in prevention of cell death and is an important regulator of protein synthesis (Adams, 1998; Rotwein, 2003). IGF-I has a critical role to activate the muscle satellite (stem) cells that are required for local muscle repair (Adams, 1998). Alternative splicing of the IGF-I gene is known to generate several different variants depending on their exon sequences (Stewart & Rotwein, 1996). Yang *et al.* (1996) cloned the cDNAs of IGF-I splice variants that are expressed in muscle of rabbits and humans. These include IGF-IEa and mechano growth factor (MGF). The former is the same as the circulating IGF-IEa produced by the liver, and the latter is reported to be detected in normal muscle and is markedly upregulated after mechanical stimulation (Yang *et al.* 1996; McKoy *et al.* 1999). MGF in rodents is structurally identical to IGF-IEb, originally identified as an isoform of hepatic IGF-I (Roberts *et al.* 1987). MGF mRNA is derived from the IGF-I gene by alternative precursor mRNA splicing, the sequence of which has a 52 base pair insert in rodents within the E domain. This results in a translational frame-shift that results in a different carboxy terminal sequence to that of IGF-IEa. Previous reports suggest that the E domain of pro-IGF-I precursor may function as a unique growth factor independent of IGF-1 (Siegfried *et al.* 1992; Tian *et al.* 1999; Yang & Goldspink, 2002). IGF-IEa peptide transfected into C2C12 myoblast cells caused an increase in cell density and the myoblasts fused to form myotubes whereas MGF peptide caused the mononucleated myoblasts to increase in number but prevented differentiation (Yang & Goldspink, 2002). Hill & Goldspink (2003) demonstrated that MGF was rapidly

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expressed after muscle damage following the activation of muscle satellite cell and upregulation of IGF-IEa, suggesting that the initial pulse of MGF, not the IGF-IEa, was responsible for satellite cell activation in rat skeletal muscle. In addition, Aperghis *et al.* (2004) demonstrated that MGF is markedly more effective than IGF-Ia for motoneurone survival, suggesting that MGF may play a role in neuronal maintenance and survival as well as muscle satellite cell activation.

Growth hormone (GH) is the principal regulator of IGF-I expression in tissues (Clemmons *et al.* 1981; D'Ercole *et al.* 1984). Transgenic mice that express bovine GH have high serum IGF-I levels as well as high hepatic IGF-I gene expression compared with wild-type mice (Chen *et al.* 1997; Iida *et al.* 2004). In contrast, GH-deficient *lit/lit* mice or GH receptor (GHR) gene-disrupted (GHR−/−) mice have extremely reduced levels of serum IGF-I as well as low hepatic IGF-I mRNA levels (Donahue & Beamer, 1993; Zhou *et al.* 1997). There is *in vitro* evidence for the regulation of IGF-I by GH in a mouse C2C12 myoblast cell line (Sadowski *et al.* 2001; Frost *et al.* 2002). *In vivo*, hypophysectomized rats showed reduced expression of IGF-I in muscle that was restored by GH administration (Lemmey *et al.* 1997; Wilson *et al.* 1998). Therefore, we hypothesized that MGF, derived from IGF-I gene by alternative splicing, might be regulated not only by exercise or damage as reported previously, but also by GH in skeletal muscle. Ageing in humans is characterized by a loss of muscle strength and mass, and also by a gradual decline in circulating GH (Rudman *et al.* 1990). It is possible that sarcopenia may be linked to impairment of satellite cell activation by IGF-I, especially by MGF (Chakravarthy *et al.* 2000). If a chronic state of GH deficiency is associated with reduced levels of MGF, it is compatible with the phenotype of reduced muscle mass observed in elderly people or *lit/lit* mice. Recently, Hameed *et al.* (2004) reported that MGF mRNA was significantly increased in healthy elderly men after treatment for 12 weeks with recombinant GH. The same study demonstrated that MGF mRNA was not changed whereas IGF-IEa mRNA was significantly increased after treatment for 5 weeks with recombinant GH, suggesting that IGF-I isoforms in muscle were differentially regulated by GH. However, the effects of chronic GH excess or deficiency on regulation of MGF mRNA, or acute effects of GH on expression of different splice variants of the IGF-I gene remain to be characterized.

The aim of this study was to: (1) determine the effect of chronic GH excess or disruption of GHR signalling on expression of IGF-I isoforms in skeletal muscle; (2) determine the acute effect of a physiological or slightly supraphysiological dose of GH on expression of IGF-I isoforms in muscle of GH-deficient *lit/lit* mice or GH-sufficient *lit/+* mice; and (3) examine the differences in regulation by GH of expression of IGF-I gene isoforms in muscle compared to liver.

Methods

Animals and tissues

All studies were performed using 3-month-old male mice. Two different strains of mice, referred to as bGH (giant transgenic mice that express bovine GH) and GHR−/− (dwarf GH receptor/binding protein gene-disrupted mice) were used to examine the chronic effect of excessive or deficient GH action. Non-transgenic or non-disrupted littermates were used as controls, respectively. These mouse colonies were generated and maintained at Ohio University. The production and characterization of bGH transgenic and GHR−/− mice were described in detail previously (Chen *et al.* 1997; Zhou *et al.* 1997). The GH-deficient *lit/lit* dwarf mice with an inactivating mutation of GH-releasing hormone receptor (Godfrey *et al.* 1993) were used to examine the effect of chronic isolated GH deficiency and the acute response to GH treatment. The *lit/+* mice (GH-sufficient) were used as controls. C57BL/6 J *lit/lit* and *lit/+* mice were produced at a customized colony at the Jackson Laboratory (Bar Harbour, ME, USA). A multiple control experimental design was used, that included: (1) vehicle-treated *lit/lit* mice, reflecting profound chronic GH deficiency; (2) GH-treated *lit/lit* mice, reflecting acute changes in response to a single dose of GH; (3) vehicle-treated *lit/+* mice, reflecting the normal pattern of GH in terms of both magnitude and pulsatility (Donahue & Beamer, 1993); and (4) GH-treated *lit/+* mice, reflecting at least physiological, if not supraphysiological effects of GH. Mice ($n = 5$ in each group) received a s.c. bolus (120 ng (g body weight)$^{-1}$) of rat recombinant GH (rrGH) (Genentech Inc., South San Francisco, CA, USA) or vehicle (0.9% saline, 100 μl) 4 h and 12 h before anaesthesia with halothane and were killed for tissue harvesting. All mice were allowed to move freely. Food and water were supplied *ad libitum*. Liver and skeletal muscle (gastrocnemius) were collected and flash-frozen in liquid nitrogen and stored at −80°C for subsequent mRNA analysis. All animal experiments were approved by the Animal Care and Use Committees of the University of Virginia or Ohio.

Total RNA preparations

The RNA extraction was performed using Tri Reagent (Molecular Research Center, Inc. Cincinnati, OH, USA) followed by RNeasy Mini Kit (Qiagen, Inc. Valencia, CA, USA) according to the manufactures' instructions. The quantity of extracted total RNA was determined using the RiboGreen RNA Quantification Kit (Molecular Probes,

Eugene, OR, USA) with a Genios multidetection reader (Phenix Research Product, Hayward, CA, USA).

Real-time RT-PCR

Primers for murine IGF-IEa and IGF-IEb were designed as previously described (Hill & Goldspink, 2003) with modifications (Fig. 1*A*). The 18S rRNA primers were as previously described (Chen & Hughes-Fulford, 2001). The PCR primers used were as follows: IGF-IEa, forward, 5′-GCTTGCTCACCTTCACCAGC-3′, reverse, 5′-AATGTACTTCCTTCTGAGTCT-3′; IGF-IEb, forward, 5′-GCTTGCTCACCTTCACCAGC-3′, reverse, 5′-AAATGTACTTCCTTTCCTTCTC-3′; 18S rRNA, forward, 5′-TCAAGAACGAAAGTCGGAGG-3′, reverse, 5′-GGACATCTAAGGGCATCACA-3′. The reaction of RT-PCR was previously described (Iida *et al.* 2004). Briefly, 500 ng total RNA from liver and skeletal muscle was reverse transcribed in a total volume of 10 μl using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacture's instruction. A 1 : 20 dilution of the resultant cDNA was prepared and 4 μl of this template was used in the real-time PCR protocol. The iCycler iQ Real-Time PCR detection system (Bio-Rad Laboratories, Inc. Hercules, CA, USA) was used for sample cDNA quantification. Real-time quantitative PCR was performed in a volume of 20 μl using JumpStart Taq DNA Polymerase (Sigma, St Louis, MO, USA) and SYBR Green I (Molecular Probes) was used for detection of PCR products. The PCR protocol consisted of 5 min at 95°C followed by 40 cycles of 15 s at 94°C, 40 s at 55°C, and 45 s at 72°C. To assess PCR specificity, melting curves from 55 to 95°C in 0.5°C steps of 10 s with measurement of fluorescence

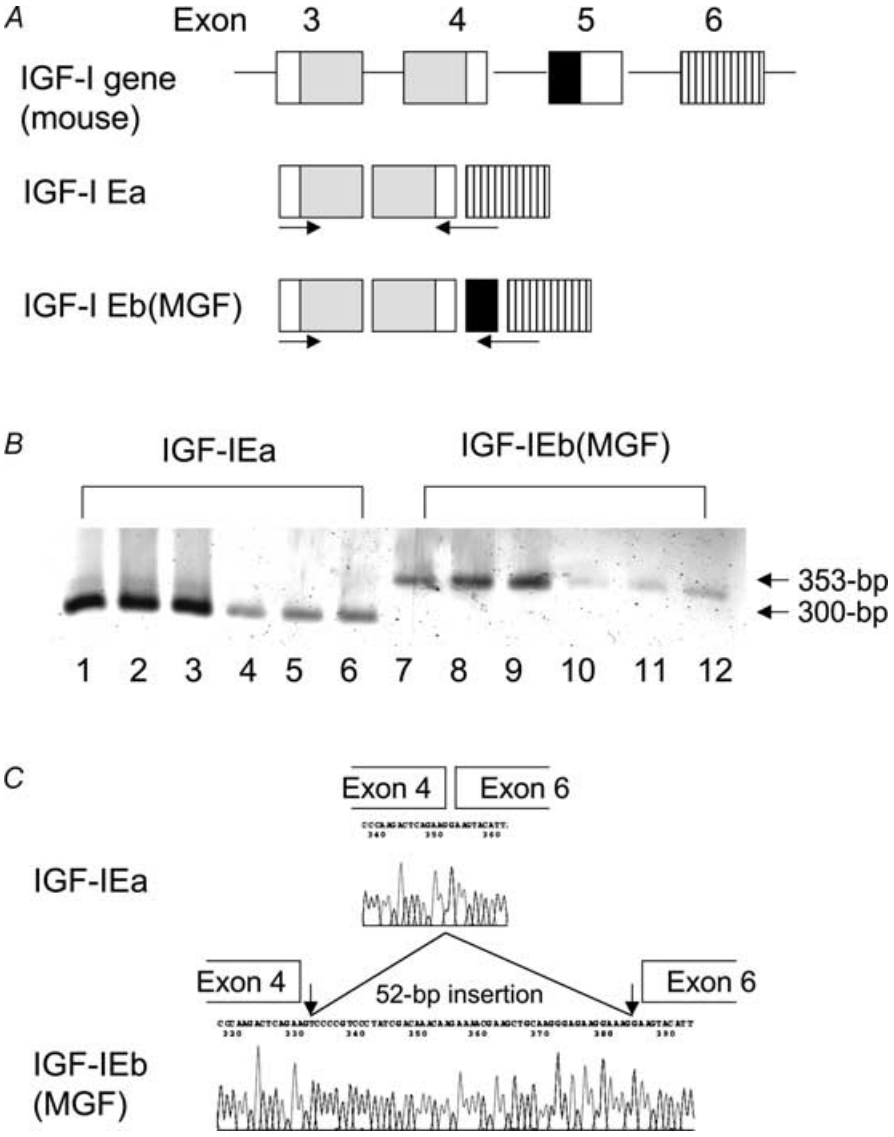

Figure 1. Detection of IGF-IEa and MGF mRNA in skeletal muscle
A, alternative splicing process producing murine IGF-I isoforms and the position of primers used in this study. The grey box indicates the coding region of mature IGF-I protein. The black box indicates the 52-base pair nucleotides that are inserted in IGF-IEb protein. *B*, detection of both IGF-IEa and IGF-IEb (MGF) in skeletal muscle of wild-type mice (lane: 1–3 and 7–9) and GHR−/− mice (lane: 4–6 and 10–12). Amplified products by RT-PCR were separated on 2% agarose gel and visualized with ethidium bromide staining. *C*, sequence analysis of the band shown in *B* confirmed that the amplified products were IGF-IEa and IGF-IEb (MGF), respectively.

were generated at the end of each PCR. A single melting peak and a single band on 2% agarose gel electrophoresis were confirmed for each gene product. The nucleotides of PCR products were confirmed by sequencing using a DNA sequencer (model 3100, Applied Biosystems, Foster City, CA, USA).

Quantification

Plasmids including the PCR product of murine IGF-IEa, IGF-IEb or 18S rRNA were constructed, and the DNA concentration of each plasmid was determined using a Biomate Spectrophotometer (260 nm/280 nm) (Thermo Spectronic, Rochester, NY, USA). These plasmids were used as standards for quantification. A standard curve was generated by amplifying serial dilutions of a known quantity of plasmid DNA. The standards and cDNA samples were then co-amplified in the same reaction plate. The standard curve displayed a linear relationship between cycle threshold (Ct) values and the logarithm of input plasmid copy number. The amount of product in a particular sample was determined by interpolation from a standard curve of Ct values generated from the plasmid dilution series. All measurements were performed in triplicate in individual assays and the mean value of the triplicate was used for analysis.

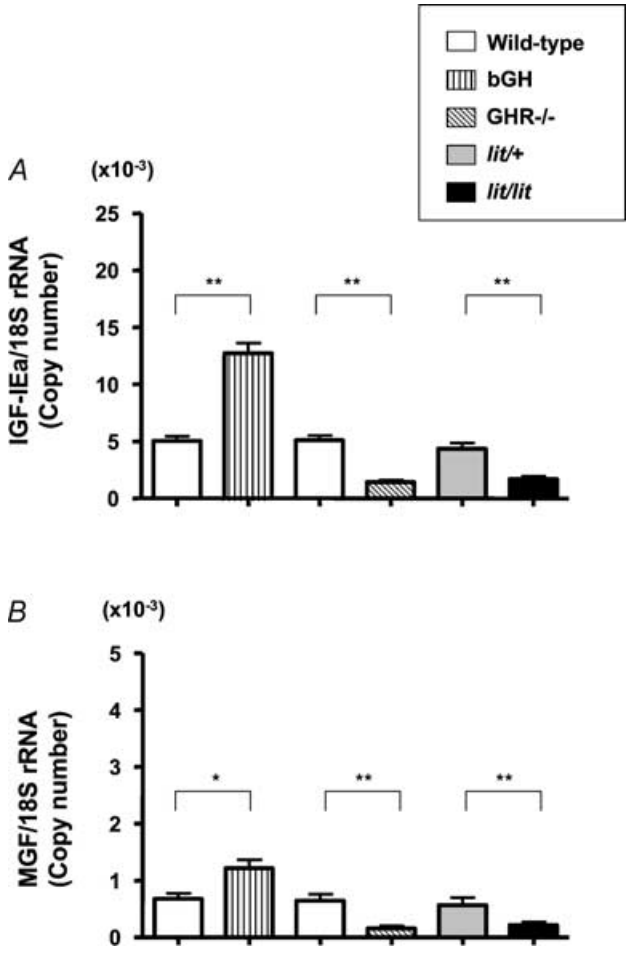

Figure 2. Expression of IGF-IEa and MGF mRNA in skeletal muscle

Expression of IGF-IEa (*A*) and MGF (*B*) mRNA in skeletal muscle in bGH, GHR−/− and *lit/lit* mice. Non-transgenic or non-knockout littermates were used as controls for bGH or GHR−/−, respectively. *lit/+* mice were used as controls for *lit/lit* mice; $n = 6$ in each group. $^{*}P < 0.05$; $^{**}P < 0.01$.

Data analysis

Results of the expression of isoforms of the IGF-I gene were adjusted to18S rRNA amplified, and were expressed as mean ± s.e.m. Differences were determined by unpaired *t* test or one-way ANOVA, as appropriate. $P < 0.05$ was considered significant.

Results

Mice were weighed before injection of GH or vehicle. The bGH mice weighed 42.7 ± 3.9 g (mean ± s.d.) whereas the control mice weighed 29.9 ± 2.4 g ($P < 0.0005$). The GHR−/− mice weighed 19.4 ± 3.3 g whereas the control mice weighed 32.1 ± 2.7 g ($P < 0.001$). The *lit/lit* mice weighed 17.1 ± 1.8 g whereas *lit/+* mice weighed 30.4 ± 1.2 g ($P < 0.001$).

Using the specific primer for IGF-IEa and MGF (IGF-IEb), the RT-PCR products from skeletal muscle of GHR−/− as well as wild-type mice were detected (Fig. 1*B*). These products were confirmed to be IGF-IEa and MGF, respectively, by sequencing (Fig. 1*C*).

In this study, 18S rRNA was used as a housekeeping gene as our preliminary study showed the consistency of 18S rRNA irrespective of altered GH action or treatment by GH whereas the amount of other housekeeping genes such as GAPDH or β-actin was changed by GH treatment (data not shown). As shown in Fig. 2*A*, IGF-IEa mRNA in skeletal muscle in bGH mice was 266% and that in GHR−/− mice was 30% of that in control mice. IGF-IEa mRNA in skeletal muscle in *lit/lit* mice was 39% of that in *lit/+* mice. On the other hand, MGF mRNA in skeletal muscle in bGH mice was 152% and that in GHR−/− mice was 19% of that in control mice. MGF mRNA in skeletal muscle in *lit/lit* mice was 25% of that in *lit/+* mice (Fig. 2*B*).

We then examined the changes of IGF-I isoforms expressed in muscle after a single injection of GH using GH-deficient (*lit/lit*) or GH-sufficient (*lit/+*) mice. Both IGF-IEa and MGF mRNAs were significantly increased (490% and 275% of control mice, respectively) 4 h after GH injection in *lit/+* mice. On the other hand, IGF-IEa mRNA was not increased whereas MGF was significantly increased (553% of control mice) 4 h after GH injection in *lit/lit* mice (Fig. 3*A* and *B*). In *lit/+* mice, both IGF-IEa and MGF mRNAs returned to the basal level

12 h after GH injection whereas both IGF-IEa and MGF mRNAs were significantly increased (252% and 301% of control mice, respectively) 12 h after GH injection in *lit/lit* mice (Fig. 3*C* and *D*).

Finally in the same studies we examined liver, where IGF-I mRNA is abundantly expressed, to verify the tissue-specificity of regulation of MGF expression. We confirmed that both IGF-I isoforms were expressed in liver of not only wild-type mice but also bGH and GHR−/− mice (data not shown). In contrast to the skeletal muscle, in *lit/+* mice neither IGF-IEa nor MGF mRNAs were increased whereas in *lit/lit* mice, both were significantly increased (303% and 299% of control mice, respectively) 4 h after GH treatment, and returned to basal level 12 h after GH injection (Fig. 4).

Discussion

The main finding of the present study is that in skeletal muscle, in addition to IGF-IEa mRNA, MGF mRNA is regulated by GH. Previous reports have emphasized the role of exercise in regulation of MGF mRNA expression in skeletal muscle (Yang *et al.* 1996; McKoy *et al.* 1999). Of more importance, we found that a single injection of GH increased MGF mRNA more rapidly than IGF-IEa mRNA in skeletal muscle of GH-deficient mice. Furthermore, the kinetics of MGF and IGF-IEa in skeletal muscle after GH treatment were different depending on whether the mouse was GH-deficient or not.

There is some evidence that IGF-IEb is regulated by GH (Roberts *et al.* 1987; Lowe *et al.* 1988). However, the previous report focused on hepatic IGF-IEb regulation by GH using hypophysectomized rats with four daily high dose injections of purified rat GH (150 μg, I.P) (Lowe *et al.* 1988). The advantages and differences of our study from previous reports are that: (1) mouse models with isolated GH deficiency, completely disrupted GHR signalling, or chronic GH excess were used; (2) we focused on skeletal muscle to elucidate the role of IGF-IEb as 'mechano growth factor'; (3) we examined the acute effect of GH treatment in GH-deficient and GH-sufficient mice; and (4) we used a lower, more physiological dose of rrGH (120 ng (g body weight)$^{-1}$, i.e. < 4 μg per injection for control animals) than that used in the previous study (150 μg per injection, i.e > 500 ng (g body weight)$^{-1}$) (Lowe *et al.* 1988) in order to mimic as closely as possible the physiological state (Waxman *et al.* 1991; Kalu *et al.* 1998). That the dose was close to physiological levels is supported by the lack of effect in liver of the GH-sufficient *lit/+* mice to this dose of rrGH in contrast to the large effect observed in the GH-deficient *lit/lit* mice (Fig. 4).

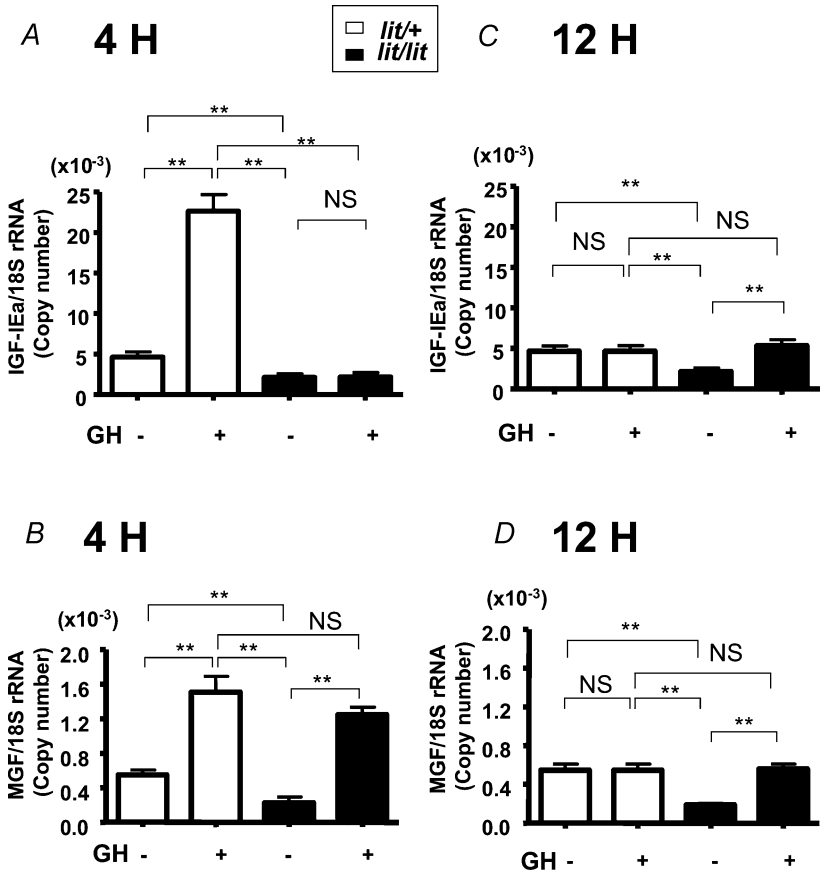

Figure 3. Expression of IGF-IEa and MGF mRNA in skeletal muscle
Expression of IGF-IEa (*A* and *C*) and MGF (*B* and *D*) mRNA in skeletal muscle of *lit/+* mice or *lit/lit* mice 4 h (*A* and *B*) or 12 h (*C* and *D*) after GH- or vehicle-treatment; $n = 5$ in each group.
$^{**}P < 0.01$; NS, not significant.

Our results using bGH and GHR−/− mice suggest that MGF as well as IGF-IEa mRNA levels in skeletal muscle paralleled GH action in the chronic state (Fig. 2). On the other hand, detection of MGF and IGF-IEa mRNA in skeletal muscle of GHR−/− mice indicates that GH is not the exclusive regulator of MGF and IGF-IEa in skeletal muscle as previously suggested (DeVol *et al.* 1990). Both MGF and IGF-IEa mRNAs were detected in GHR−/− mice in liver as well (data not shown). It is well known that ageing is associated with reduced serum GH levels and with reduced muscle mass. If MGF plays a relevant role to activate muscle satellite cells as demonstrated by Yang & Goldspink (2002), our results suggest that reduced serum GH levels associated with ageing might play a role in this reduction of muscle mass. We speculate that low MGF mRNA expression in the basal state in GH deficiency may be associated with a relative resistance to exercise-induced MGF expression. GH may be crucial to facilitate the increase of MGF by exercise as discussed by Hameed *et al.* (2004). Supporting our present data, lower expression of MGF mRNA in old rats was observed compared with that in young rats before and after muscle overload (Owino *et al.* 2001). However, no difference was observed in the basal expression level of MGF in muscle between young and older people although there was a significant difference between these groups after exercise (Hameed *et al.* 2003). The reason for the discrepancy between rodents and humans is unclear. However one possible explanation is that muscle strength varies in older people depending on life-style, and non-GH factors (i.e. habitual exercise) may compensate for the reduced GH-associated MGF mRNA expression in some older people.

A relevant finding in this study is that MGF mRNA in skeletal muscle increased more rapidly than IGF-IEa after GH treatment in GH-deficient *lit/lit* mice (Fig. 3). On the other hand, IGF-IEa mRNA was already increased 4 h after GH treatment in GH-sufficient *lit/+* mice (Fig. 3*A* and *B*). Taken together, MGF mRNA is preferentially produced after GH treatment in skeletal muscle in the chronic GH-deficient state. As mice used in this study were physically active, we are unable to exclude the possibility that activity status plays a role in regulating the splicing of the IGF-I gene after upregulation of primary IGF-I transcript by GH administration. However, the significant differences of MGF mRNA induction between GH-treated and vehicle-treated *lit/lit* mice despite all mice being physically active suggest that GH may have a potential role to regulate pre-mRNA splicing of the IGF-I gene in skeletal muscle. There is evidence that MGF has a different physiological role in skeletal

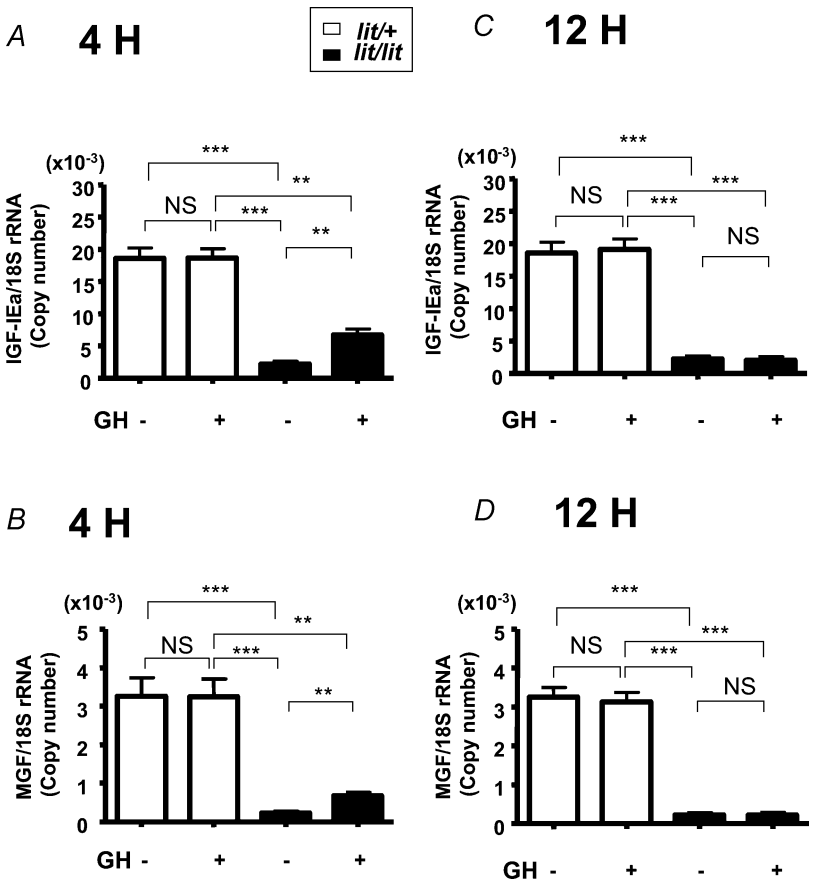

Figure 4. Expression of IGF-IEa and MGF mRNA in liver
Expression of IGF-IEa (*A* and *C*) and MGF (*B* and *D*) mRNA in liver of *lit/+* mice or *lit/lit* mice 4 h (*A* and *B*) or 12 h (*C* and *D*) after GH- or vehicle-treatment; $n = 5$ in each group. $^{**}P < 0.01$; $^{***}P < 0.001$; NS, not significant.

muscle from IGF-IEa. Yang & Goldspink (2002) reported that the distinct E domain of MGF inhibits terminal differentiation while increasing myoblast proliferation, which contrasts with the role of mature IGF-I peptide. Furthermore, Shavlakadze *et al.* (2004) demonstrated that transgenic mice with skeletal muscle-specific over-expression of IGF-IEa did not stimulate the early events associated with myogenesis, suggesting that other IGF-I isoforms such as MGF rather than IGF-IEa may play a relevant role in the early events of tissue repair in skeletal muscle. Thus, preferential induction of MGF mRNA by GH replacement in the situation of GH deficiency may be favourable for the efficient activation of muscle satellite cells although we did not measure the protein level of MGF.

Another interesting finding in this study is that the timing of IGF-I mRNA expression was different between liver and skeletal muscle despite the fact that a s.c injection was not expected to affect preferentially any of these tissues. Four hours after GH administration in *lit/lit* mice, both IGF-IEa and MGF were increased in liver (Fig. 4*A* and *B*) whereas only MGF was increased in skeletal muscle (Fig. 3*A* and *B*). On the other hand 12 h after GH treatment, both hepatic IGF-IEa and MGF mRNA levels returned to the basal level (Fig. 4*C* and *D*) whereas both IGF-IEa and MGF mRNA levels were increased in skeletal muscle (Fig. 3*C* and *D*). This suggests a faster mRNA turnover in liver, that might be related to a tighter regulation of IGF-I production and secretion, as the liver is the main source of the IGF-I protein in serum that regulates pituitary GH secretion (Yakar *et al.* 1999; Sjogren *et al.* 1999; Wallenius *et al.* 2001). Alternatively, it might reflect an overall faster mRNA turnover in liver than in skeletal muscle, affecting a variety of genes besides IGF-I. There were no differences in hepatic IGF-IEa or MGF mRNA levels between GH-treated and vehicle-treated *lit/+* mice either 4 h or 12 h after treatment (Fig. 4) whereas in skeletal muscle, both IGF-IEa and MGF mRNA levels were significantly increased in *lit/+* mice 4 h after GH treatment (Fig. 3*A* and *B*) and returned to the basal level 12 h after treatment (Fig. 3*C* and *D*). These results show a different timing of induction of IGF-I mRNA by GH between liver and skeletal muscle; muscle is a slow responder to GH *in vivo*. Sadowski *et al.* (2001) demonstrated that the activation of mitogen-activated protein kinase (MAPK) or phosphatidylinositol 3-phosphate kinase (PI3K) induced by GH inhibited, rather than stimulated, IGF-I mRNA expression in C2C12 myoblast cell lines. Frost *et al.* (2002) demonstrated that signal transducer and activator of transcription (Stat) 3 played a critical role in GH-induced IGF-I mRNA induction in C2C12 myoblast cells, in contrast to the results observed in hepatocytes (Ram *et al.* 1996). These results suggest that the difference of post receptor pathways involved in GHR signalling in a given tissue may explain the tissue-specific difference of induction of IGF-I mRNA. We did not find that hepatic MGF was increased more rapidly than hepatic IGF-IEa. The rrGH dose used in this studies might have been low enough to induce detectable changes in liver in GH-sufficient *lit/+* mice. Alternatively, an increase in IGF-IEa earlier than 4 h and/or MGF mRNA in liver returning quickly to the basal level may have been missed. Considering that physiologically hepatic IGF-I is secreted in the circulation and that it accounts for > 75% of serum IGF-I (Yakar *et al.* 1999; Sjogren *et al.* 1999), the physiological significance of hepatic MGF is unclear but may be different from that of skeletal muscle.

In conclusion, we have demonstrated that MGF expression in skeletal muscle is associated with GH action *in vivo*. In a GH-deficient state, MGF mRNA is increased by GH administration more rapidly than IGF-IEa whereas this is not observed in the GH-sufficient state. The induction of IGF-I mRNA expression by GH is tissue-specific.

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