

## Short-term strength training and the expression of myostatin and IGF-I isoforms in rat muscle and tendon: differential effects of specific contraction types

K. M. Heinemeier,<sup>1\*</sup> J. L. Olesen,<sup>1\*</sup> P. Schjerling,<sup>3,4</sup> F. Haddad,<sup>2</sup>  
H. Langberg,<sup>1</sup> K. M. Baldwin,<sup>2</sup> and M. Kjaer<sup>1</sup>

<sup>1</sup>*Institute of Sports Medicine, Bispebjerg Hospital, Copenhagen;* <sup>2</sup>*Department of Physiology and Biophysics, University of California, Irvine, California;* <sup>3</sup>*Department of Molecular Muscle Biology, Copenhagen Muscle Research Centre, Rigshospitalet, Copenhagen;* and <sup>4</sup>*Department of Medical Biochemistry and Genetics, University of Copenhagen, Copenhagen Denmark*

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**Heinemeier KM, Olesen JL, Schjerling P, Haddad F, Langberg H, Baldwin KM, Kjaer M.** Short-term strength training and the expression of myostatin and IGF-I isoforms in rat muscle and tendon: differential effects of specific contraction types. *J Appl Physiol* 102: 573–581, 2007. First published October 12, 2006; doi:10.1152/jappphysiol.00866.2006.—In skeletal muscle, an increased expression of insulin like growth factor-I isoforms IGF-IEa and mechano-growth factor (MGF) combined with downregulation of myostatin is thought to be essential for training-induced hypertrophy. However, the specific effects of different contraction types on regulation of these factors in muscle are still unclear, and in tendon the functions of myostatin, IGF-IEa, and MGF in relation to training are unknown. Female Sprague-Dawley rats were subjected to 4 days of concentric, eccentric, or isometric training ( $n = 7$ –9 per group) of the medial gastrocnemius, by stimulation of the sciatic nerve during general anesthesia. mRNA levels for myostatin, IGF-IEa, and MGF in muscle and Achilles' tendon were measured by real-time RT-PCR. Muscle myostatin mRNA decreased in response to all types of training (2- to 8-fold) ( $P < 0.05$ ), but the effect of eccentric training was greater than concentric and isometric training ( $P < 0.05$ ). In tendon, myostatin mRNA was detected, but no changes were seen after exercise. IGF-IEa and MGF increased in muscle (up to 15-fold) and tendon (up to 4-fold) in response to training ( $P < 0.01$ ). In tendon no difference was seen between training types, but in muscle the effect of eccentric training was greater than concentric training for both IGF-IEa and MGF ( $P < 0.05$ ), and for IGF-IEa isometric training had greater effect than concentric ( $P < 0.05$ ). The results indicate a possible role for IGF-IEa and MGF in adaptation of tendon to training, and the combined changes in myostatin and IGF-IEa/MGF expression could explain the important effect of eccentric actions for muscle hypertrophy.

insulin-like growth factor-I; skeletal muscle; eccentric loading

MUSCLE GROWTH AND ADAPTATION of the supporting connective tissue in adults is dependent on a balance of negative and positive regulators of protein synthesis. Myostatin and insulin like growth factor-I (IGF-I) are regarded as two of the major players in regulation of muscle homeostasis (16, 33). They have contrasting effects on muscle growth, and regulation of their expression is suggested to be crucial in relation to exercise-induced muscle hypertrophy. Although evidence suggests

adaptive changes in tendon in response to exercise (8, 31, 32, 39, 48), the role of myostatin and IGF-I in connective tissue is largely unknown.

Myostatin, a member of the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily, is a negative regulator of muscle growth, and the absence of functional myostatin is associated with hypermuscular phenotypes in mice and cattle (36, 37). In vitro studies indicate that myostatin inhibits myoblast proliferation during myogenesis (53) as well as satellite cell activation and protein synthesis in adult mouse muscle cells (35, 52). In addition, a number of studies show a decrease in myostatin expression in response to both long- and short-term resistance training, as well as acute resistance exercise, in humans, and in response to short-term resistance and endurance training in rats (17, 18, 26, 27, 34, 47, 49). These observations point to myostatin as an important regulator of muscle growth, and a reduction in myostatin expression could be essential for exercise-induced hypertrophy of skeletal muscle. However, the exact role of myostatin in relation to different types of training, e.g., concentric vs. eccentric contractions, is unknown, and likewise nothing is known regarding its role in regulation of the force-transmitting tendon tissue.

In contrast to myostatin, IGF-I is a positive regulator of muscle growth. It appears to act on several levels, including satellite cell activation (5), gene transcription, and protein translation (6), and seems essential in mediating the loading-induced hypertrophy of skeletal muscle (15). Different splice variants of IGF-I are expressed in muscle tissue (20, 44), and whereas IGF-IEa (liver-type IGF-I) is produced both in liver and muscle tissue, the splice variant IGF-IEb (mechano-growth factor; MGF) is thought to be expressed mostly as a local growth factor in skeletal muscle (15). The expression of both IGF-IEa and MGF is increased in response to several types of muscle loading (4, 7, 10, 11, 16, 17, 20, 24). This increased expression has been shown to persist during long-term resistance training in humans (19) but seems to be initiated already in the early phase of long-term functional loading in rat muscle (4, 11, 56) and has also been observed after a single resistance exercise bout in rats (17). Recent studies show a more rapid increase in MGF expression, compared with IGF-IEa, in rat muscle in response to stimulated isometric contractions (17)

\* K. M. Heinemeier and J. L. Olesen contributed equally to this study.

Address for reprint requests and other correspondence: K. M. Heinemeier, Institute of Sports Medicine, Bispebjerg Hospital-Bldg. 8, 1st Floor, 23 Bispebjerg Bakke, DK-2400 Copenhagen NV, Denmark (e-mail: katjaheinemeier@hotmail.com).

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and stretch combined with stimulation (17, 24), suggesting that the two isoforms could influence different temporal stages of muscle hypertrophy. This is supported by *in vitro* observations on mouse myoblasts, which indicate that MGF induces myoblast proliferation, but not differentiation, while IGF-IEa seems important for differentiation of myoblasts into myotubes (58). The role of IGF-IEa and MGF in relation to muscle adaptation to loading has received a great deal of attention. However, it is still unclear whether different contraction types have different effects on the expression of the IGF-I isoforms, as the few studies, which have addressed this subject, show conflicting results (3, 7). In the present study, we have investigated the specific effects of different contraction types on IGF-IEa/MGF expression.

With regard to tendon tissue, the role of IGF-I in relation to exercise/training-induced adaptation has not been described. However, IGF-I is known to induce collagen synthesis in tendon cells (1, 2), and both IGF-I protein and mRNA have been localized in tendon tissue from humans (42) and several animal species (40). Thus IGF-I could mediate the increase in collagen synthesis observed in response to exercise/training (31, 32, 39). No distinction has been made between the IGF-I mRNA splice variants in tendon in earlier studies, but recently we have found both IGF-IEa and MGF mRNA to be present in rat tendon (43). With regard to myostatin, no evidence exists on its presence in tendon.

Our aim was to investigate whether myostatin is expressed in tendon tissue and to test if the expression of myostatin, IGF-IEa, and MGF in tendon is changed in response to short-term training. Additionally, we compared the effects of short-term concentric, eccentric, and isometric training on the expression of these substances in both muscle and tendon. No data exist to our knowledge on any differential effects of contraction types on tendon adaptation, whereas several studies indicate that long-term resistance training, which includes an eccentric component, has a superior effect on muscle hypertrophy in both men and women (14, 21, 23, 25). As myostatin, IGF-IEa, and MGF are known to be important for regulation of muscle growth, it is hypothesized that eccentric loading has a greater potential than concentric and isometric loading for changing the expression of these factors in skeletal muscle and potentially also in tendon tissue.

## MATERIALS AND METHODS

**Animals.** Young adult female Sprague-Dawley rats, weighing  $238 \pm 2$  (mean  $\pm$  SE) g, were assigned randomly to three groups (minimum 7 in each group). Each group was subjected to involuntary concentric training (Con), eccentric training (Ecc), or isometric training (Iso). Rats were housed in groups in standard vivarium cages on a 12:12-h light-dark cycle and had *ad libitum* access to standard rat chow and water. The study was conducted according to the American Physiological Society's "Guiding Principles in the Care and Use of Animals," and the protocol was approved by the University of California Institutional Animal Care and Use Committee.

**Training protocol.** The model used in the present study for training of rats was identical to the model employed previously by Adams et al. (3). However, it should be noted that the number of repetitions per set and the number of training sessions in the present study were not identical to those of the study of Adams et al.

**Electrical muscle stimulation.** Before each training bout, the rats were lightly anesthetized with ketamine (30 mg/kg), xylazine (4 mg/kg), and acepromazine (1 mg/kg). Stainless steel wire electrodes,

coated with Teflon, were used for stimulation. The electrodes were introduced subcutaneously in the region adjacent to the popliteal fossa, via 22-gauge hypodermic needles (which were withdrawn, leaving the electrode in place). Before insertion, a section of the Teflon was removed, leaving the wire exposed in the area lateral and medial of the sciatic nerve and allowing for field stimulation of the nerve. The electrodes were attached to the output poles of a Grass stimulus isolation unit interfaced with a Grass S8 stimulator. This allowed for delivery of current to the sciatic nerve and thus muscle activation.

When stimulation electrodes were in place, the rats were positioned in a specially designed training platform described previously (9). The right leg was positioned in a footplate, which was attached to the shaft of a Cambridge model H ergometer. The voltage and stimulation frequency ( $\sim 50$  Hz) were adjusted to produce maximal isometric tension.

**Training.** During all training bouts the sciatic nerve of the right leg was stimulated for 2 s with 18 s between stimulations. Sets consisted of 10 stimulations and were separated by 5 min rest. Rats were trained for 4 consecutive days with two sets on *day 1*, three sets on *day 2*, and four sets on *days 3* and *4*. After each training session the electrodes were removed. The left leg served as control.

The training protocols were controlled by computer via a digital-to-analog board (model DDA-06, Keithley Instruments) used to control footplate movement and to trigger the stimulus. A separate analog-to-digital board (DAS-16) was used to acquire force measurements (100-Hz acquisition). Data acquisition, control of stimulus triggering, and footplate movements were programmed by using LabTech Note-book (Laboratory Technologies). Data analysis was conducted by using Acqknowledge software (Biopac Systems). Force output was monitored in real time on the computer screen during each contraction.

Rats of the Iso group had their right foot placed in the footplate with an angle of  $\sim 44^\circ$  relative to the tibia. The footplate angle was fixed during muscle stimulation. For the Con group, the ergometer allowed the footplate to move from  $44^\circ$  to  $64^\circ$  after the development of maximal isometric tension in the beginning of muscle contraction. For the Ecc group, the footplate was moved from  $44^\circ$  to  $24^\circ$  after the development of maximal isometric tension. The movement rate was limited to  $29^\circ/\text{s}$  in the Con and Ecc groups to maintain force development.

The choice of the present short-term training protocol was based on previous observations by Adams et al. (3), who found that 10 days of training spread over a 20-day period resulted in muscle hypertrophy and increased IGF-I mRNA, combined with the findings of Haddad and Adams (17), which indicated that many of the signals for protein synthesis are turned on after only two training sessions. It should be noted that muscles trained under these conditions generate forces that are significantly different among the three training modes, i.e., eccentric-trained muscles generated the most force, while the concentric-trained muscles generated the least amount of force (3).

**Tissue sampling and storage.** Twenty-four hours after the last training bout, the rats were killed via an injection of Pentosol euthanasia solution (Med-Pharmex) at a dose of 0.4 ml/kg. The medial gastrocnemius including the Achilles tendon, from both legs, was dissected free, and the midsection of the muscle belly and the Achilles tendon were cut out and stored at  $-80^\circ\text{C}$  for later use.

**RNA extraction.** Total RNA from all tissue types was extracted according to the method described by Chomczynski and Sacchi (9a). Muscle tissue was homogenized in TRI-reagent (MRC) with a Polytron homogenizer (Ultra-Turrax T8, Ika labortechnik, Staufen, Germany), while tendon tissue was homogenized in TRI-reagent using a bead-mixer (Retsch, MM300) with the aid of 3 mm steel beads. The tubes containing tendon tissue, TRI-reagent, and beads were shaken at 24 Hz for 60 s and then cooled on ice. This was repeated seven times.

Following homogenization, 1-bromo-3-chloropropane (BCP)

Table 1. Primers for real-time RT-PCR

mRNA	Sense Primer	Antisense Primer
Myostatin	TGCTGTAACTTCCCAGGACCA	GTGAGGGGGTAGCGACAGCAC
GAPDH	CCATTCTTCCACCTTTGATGCT	TGTTGCTGTAGCCATATTCATTGT
RPLP0	AGGGTCCTGGCTTTGTCTGTGG	AGCTGCAGGAGCAGCAGTGG
IGF-IEa	GGAGGCTGGAGATGTA CTGTGCT	TGTGTTCTTCAAGTGTACTTCCTTCTG
MGF	GGAGGCTGGAGATGTA CTGTGCT	TCCTTTCAGCTTCCTTTTCTTG

RPLP0, large ribosomal protein P0; IGF-IEa, insulin-like growth factor-I isoform Ea; MGF, mechano-growth factor.

(MRC) was added (100  $\mu$ l per 1,000  $\mu$ l TRI-reagent) to separate the samples into an aqueous and an organic phase. In tendon samples glycogen was added (120  $\mu$ g per 1,000  $\mu$ l TRI-reagent) to improve RNA precipitation. Following isolation of the aqueous phase, RNA was precipitated using isopropanol. The RNA pellet was then washed in ethanol and subsequently dissolved in RNase-free water. All samples were weighed before RNA extraction.

RNA concentrations were determined by spectroscopy at 260 nm. To account for absorbance of the glycogen added to the tendon samples, mock extractions were made in which no tissue was present, and the mean absorbance of these negative controls ( $n = 3$ ) was subtracted from all test values. RNA purity was ensured by 260/240 and 260/280 nm ratio, and RNA quality was ensured by gel electrophoresis.

**Real-time PCR.** Five hundred nanograms total RNA from muscle and 200 ng from tendon was converted into cDNA in 20  $\mu$ l using the OmniScript reverse transcriptase (Qiagen) according to the manufacturer's protocol. For each target mRNA, 0.25  $\mu$ l cDNA was amplified in a 25- $\mu$ l SYBR Green PCR reaction containing 1 $\times$  Quantitect SYBR Green Master Mix (Qiagen) and 100 nM of each primer (Table 1).

The amplification was monitored in real time using the MX3000P Real-time PCR machine (Stratagene). The cycle threshold ( $C_t$ ) values were related to a standard curve made with the cloned PCR products, and specificity was confirmed by melting curve analysis after amplification. The general range of  $C_t$  values was 15–30. The large ribosomal protein P0 (RPLP0) had been chosen as internal control, assuming RPLP0 mRNA to be constitutively expressed (12). To validate this assumption, another unrelated "constitutive" RNA, GAPDH mRNA, was measured, and RPLP0 was normalized to GAPDH. However, the RPLP0-to-GAPDH ratio was not stable, and we chose to normalize mRNA data to the weight of the tissue needed for extracting the amount of RNA used for cDNA synthesis (500 ng RNA for muscle and 200 ng RNA for tendon). The changes in RPLP0-to-GAPDH ratio are described in RESULTS. mRNA data are presented as fold changes relative to the mean of all control values for muscle or tendon.

**Number of samples in each group.** For muscle, all samples were successfully analyzed, and all groups included seven to nine samples. For tendon, five, six, and eight samples for control Con, Ecc, and Iso groups, respectively, and six, seven, and nine samples for trained Con, Ecc, and Iso groups, respectively, were included.

**Statistics.** All data [except RNA concentration (Fig. 1, I and J)] were log-transformed before statistical analyses and are presented as geometric means  $\pm$  backtransformed SE.

A two-way ANOVA on contraction  $\times$  training was performed using SAS procedure mixed with autoregressive modeling. If the two-way ANOVA was significant, individual differences between trained and untrained leg within each contraction type, and between contraction types, were tested with a post hoc test (Bonferroni). Otherwise, training effect was tested against control on all contraction types combined. Differences were considered significant when  $P \leq 0.05$ .

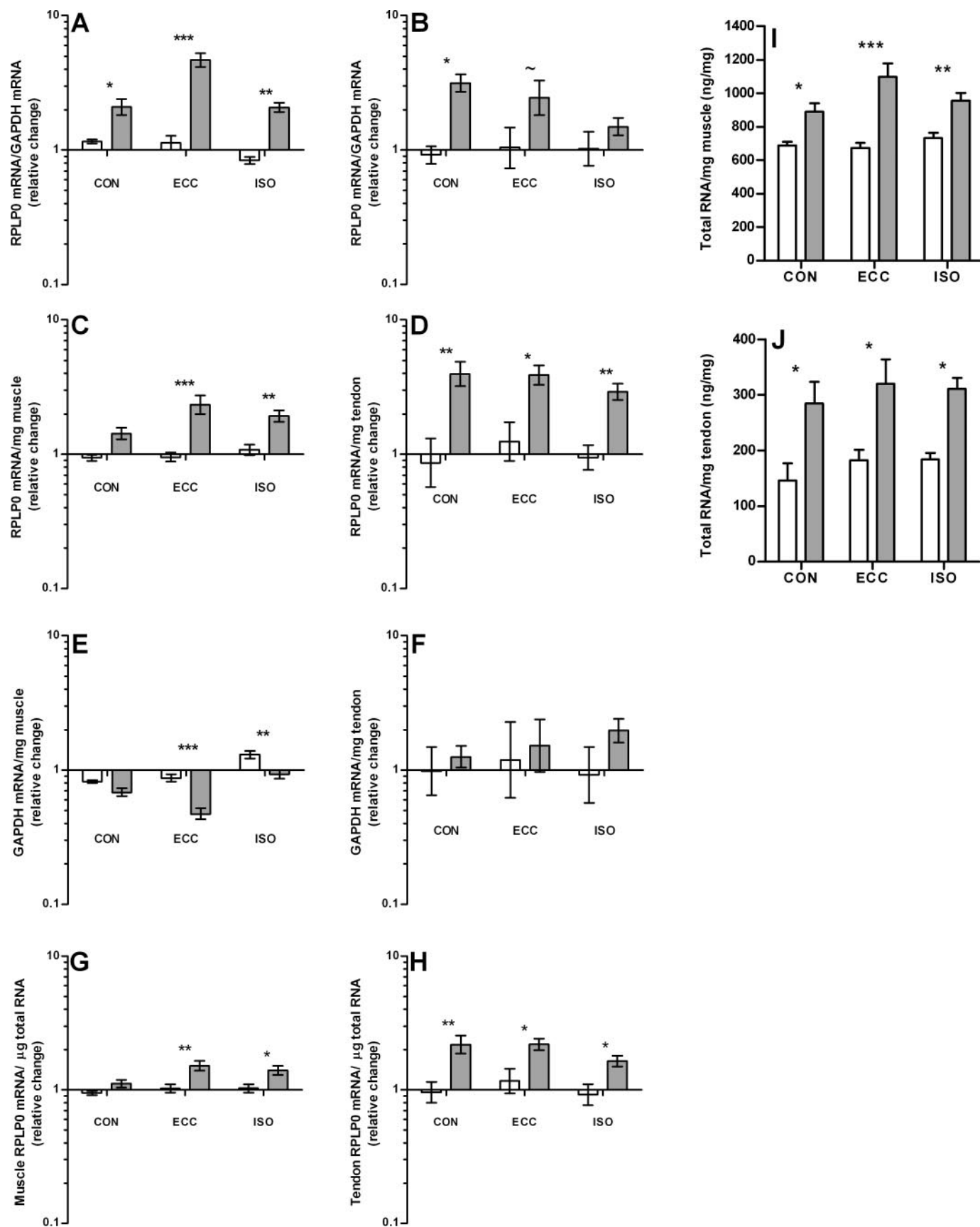
## RESULTS

**Normalization of mRNA data.** To validate the use of RPLP0 as internal reference for mRNA data, we normalized it to GAPDH, which is also assumed to be constitutively expressed. However, the RPLP0-to-GAPDH ratio was found to increase in both muscle and tendon in response to loading ( $P < 0.05$ ) (Fig. 1, A and B). When normalizing to tissue weight, we found that RPLP0 increased in both tissue types ( $P < 0.05$ ) (Fig. 1, C and D) and that GAPDH decreased in muscle tissue ( $P < 0.05$ ) (Fig. 1, E and F), which explains the marked increase in the RPLP0-to-GAPDH ratio in muscle (Fig. 1, A and B). The RNA concentration increased up to 2- and 1.6-fold, respectively, in tendon and muscle after training ( $P < 0.05$ ) (Fig. 1, I and J). This could indicate a general increase in all RNA molecules as a result of a general increase in transcriptional activity and/or an increased cell population. However, if this were the case, we would likely have found corresponding increases in all the measured mRNA species relative to tissue weight. As this was not the case (see RESULTS), it is more likely that the increase in RNA concentration reflects a general increase in the expression of ribosome elements, including ribosomal RNA and ribosomal proteins, such as RPLP0. This is supported by the pronounced changes in RPLP0 relative to tissue weight (Fig. 1, C and D). Furthermore, the RPLP0 mRNA level increased even when normalized to total RNA ( $P < 0.05$ ) (Fig. 1, G and H), and in combination with the changes observed in the RPLP0-to-GAPDH ratio this underlines the importance of investigating whether housekeeping genes are constantly expressed to validate their use as internal controls. In the present study we have to rule out the use of these housekeeping genes, and as an alternative, we have chosen to normalize all mRNA data to the weight of the tissue corresponding to the amount of RNA used for cDNA synthesis for individual samples.

**Myostatin.** Myostatin mRNA was reduced in muscle tissue in response to all types of training ( $P < 0.05$ ). Ecc training induced an  $\sim$ 8-fold decrease in myostatin mRNA ( $P < 0.001$ ), while Con and Iso training led to  $\sim$ 2- and  $\sim$ 4 fold decreases, respectively ( $P < 0.05$ ) (Fig. 2A); the effect of Ecc training was significantly greater than the effects of both Con and Iso training ( $P < 0.05$ ) (Fig. 2A). In tendon tissue, myostatin mRNA was present but at levels far lower than in muscle (relative to total RNA) (data not shown) and with large variations. No significant changes were seen in myostatin mRNA in tendon in response to training ( $P > 0.05$ ) (Fig. 2B).

**IGF-IEa.** All types of training led to significant increases in IGF-IEa mRNA ( $P < 0.01$ ) in muscle tissue, but the levels of IGF-IEa mRNA were higher after Ecc and Iso training than after Con training ( $P < 0.05$ ) (Fig. 3A). In tendon the level of





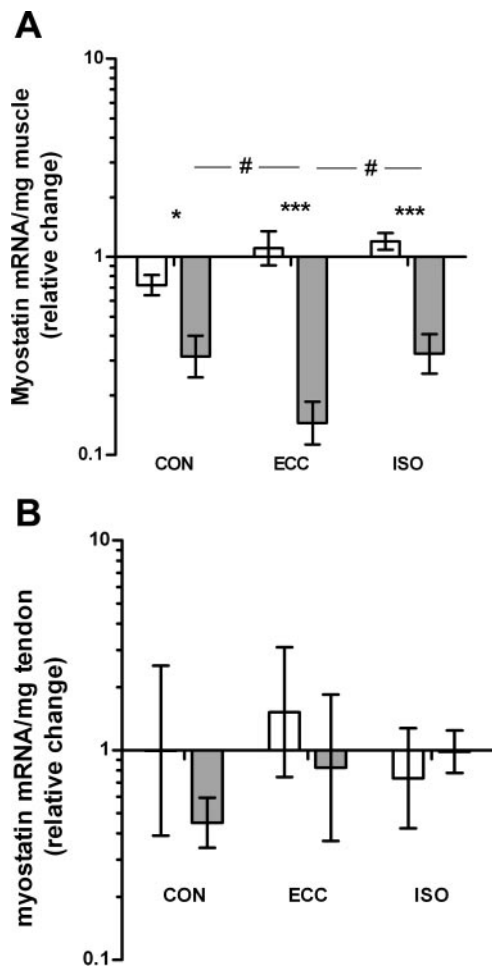


Fig. 2. Myostatin mRNA normalized to tissue weight, presented as fold changes relative to the mean of all control values, in gastrocnemius muscle (A) and Achilles tendon (B) subjected to concentric, eccentric, and isometric training (gray bars) vs. contralateral controls (open bars). Values are geometric means  $\pm$  SE. A: myostatin mRNA decreased in muscle in response to all types of training (\* $P$  < 0.05, \*\*\* $P$  < 0.001), and myostatin mRNA was lower in eccentric-trained muscle than in concentric and isometric trained (# $P$  < 0.05). B: no significant changes in myostatin mRNA in tendon ( $P$  > 0.05).

IGF-IEa mRNA (relative to total RNA) was similar to the level in muscle tissue (data not shown), and an increase was seen in response to all types of training ( $P$  < 0.05). No difference was seen between training types ( $P$  > 0.05) (Fig. 3B).

**MGF.** The regulation pattern for MGF mRNA was quite similar to that of IGF-IEa, although fold changes appeared to be higher for MGF than IGF-IEa in both muscle and tendon (Fig. 4). In muscle, MGF mRNA increased in response to all three types of training ( $P$  < 0.001) (Fig. 4A), but the effects of Ecc loading was significantly greater than the effect of Con loading ( $P$  < 0.01) (Fig. 4A). As with IGF-IEa, the level of MGF (relative to total RNA) in tendon was in a similar

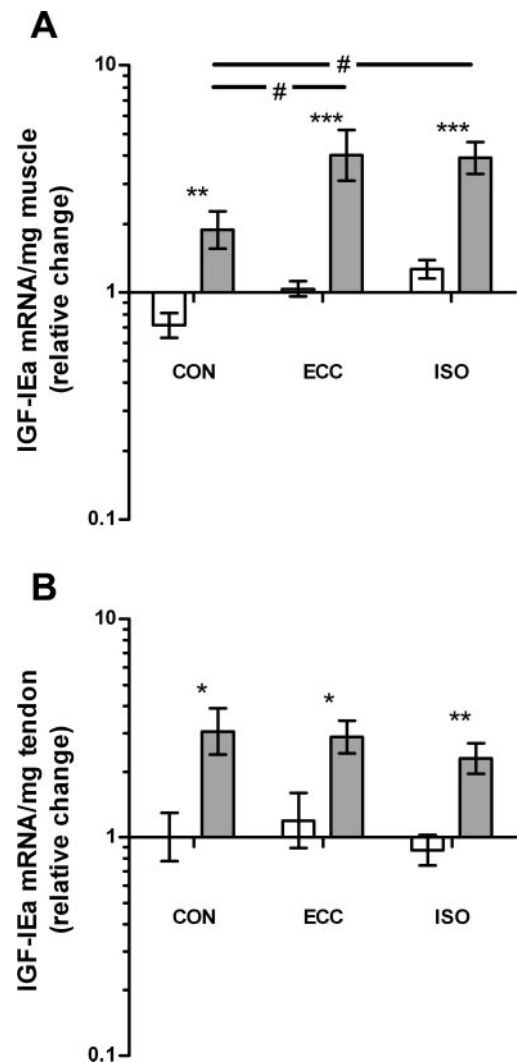


Fig. 3. Insulin-like growth factor-I isoform Ea (IGF-IEa) mRNA normalized to tissue weight, presented as fold changes relative to the mean of all control values, in gastrocnemius muscle (A) and Achilles tendon (B) subjected to concentric, eccentric, and isometric training (gray bars) vs. contralateral controls (open bars). Values are geometric means  $\pm$  SE. A: IGF-IEa mRNA increased in response to all types of training in muscle (\*\* $P$  < 0.01, \*\*\* $P$  < 0.001), and IGF-IEa was higher in eccentric- and isometric-trained muscle than in concentric trained (# $P$  < 0.05). B: IGF-IEa mRNA increased in tendon in response to all types of training (\* $P$  < 0.05, \*\* $P$  < 0.01).

range as in muscle tissue (data not shown), and an increase in MGF was seen in response to all training types ( $P$  < 0.05). No difference was seen between training types ( $P$  > 0.05) (Fig. 4B).

## DISCUSSION

The data presented here support the view that myostatin downregulation is a significant part of loading-induced muscle

Fig. 1. Training-induced changes in reference genes and RNA concentration. A and B: large ribosomal protein P0 (RPLP0) mRNA normalized to GAPDH mRNA in muscle (A) and tendon (B). C and D: RPLP0 mRNA normalized to tissue weight in muscle (C) and tendon (D). E and F: GAPDH mRNA normalized to tissue weight in muscle (E) and tendon (F). G and H: RPLP0 normalized to total RNA in muscle (G) and tendon (H). Values are presented as fold changes relative to the mean of all control values in gastrocnemius muscle and Achilles tendon subjected to concentric (Con), eccentric (Ecc), or isometric (Iso) training (gray bars) vs. contralateral controls (open bars). Values are geometric means  $\pm$  SE. \* $P$  < 0.05, \*\* $P$  < 0.01, \*\*\* $P$  < 0.001, ~ $P$  = 0.07. I and J: RNA concentration (ng/mg) in muscle (I) and tendon (J) subjected to concentric, eccentric, or isometric training (gray bars) vs. contralateral controls (open bars). Values are means  $\pm$  SE. \* $P$  < 0.05, \*\* $P$  < 0.01, \*\*\* $P$  < 0.001.

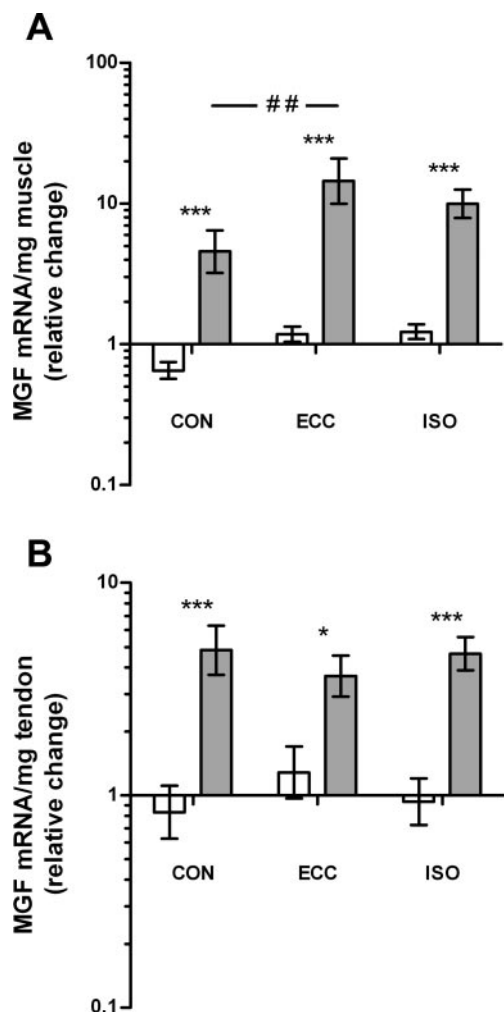


Fig. 4. Mechano-growth factor (MGF) mRNA normalized to tissue weight, presented as fold changes relative to the mean of all control values, in gastrocnemius muscle (A) and Achilles tendon (B) subjected to concentric, eccentric, and isometric training (gray bars) vs. contralateral controls (open bars). Values are geometric means  $\pm$  SE. A: MGF mRNA increased in muscle in response to all types of training ( $***P < 0.001$ ), and the level was higher in eccentric-trained muscle compared with concentric-trained muscle ( $##P < 0.01$ ). B: MGF mRNA increased in tendon in response to all types of training ( $*P < 0.05$ ,  $***P < 0.001$ ).

growth and is in line with other studies that demonstrate a decrease in myostatin mRNA levels of skeletal muscle in response to resistance type muscle loading (26, 27, 50). Furthermore, in the present study it is shown that eccentric training has a greater potential than concentric and isometric training for reducing myostatin expression in female rat skeletal muscle in the early phase of resistance training. Considering the inhibiting effect of myostatin on muscle growth, this finding could help to explain the superior effect of eccentric training on muscle hypertrophy seen in earlier studies in both men and women (14, 21, 23, 25). The only previous study describing the specific effect of eccentric loading on myostatin regulation showed a transient increase in myostatin mRNA, peaking at 3–6 h after one acute bout of muscle-damaging eccentric exercise in male rats (45). This finding appears to contradict our results and the majority of studies on myostatin that show a downregulation in response to loading (33). However, in that

study muscle was only subjected to acute exercise and not to repeated resistance training bouts, and it should be considered that the effect of one acute exercise bout differs significantly from the effect of a series of bouts with regard to expression of several other factors involved in regulation of muscle growth (17). In addition, the acute response to eccentric muscle loading could entail a transient increase in myostatin expression. Such an acute effect might also explain the increase in myostatin mRNA found in untrained young men in response to long-term heavy resistance training by Willoughby (54), as he obtained muscle samples immediately after the last training bout. Meanwhile, a very recent study shows a decrease in muscle levels of myostatin mRNA only 4 h after a moderate bout of resistance exercise in young and old women (47), and thus it must be acknowledged that regulation of myostatin in response to acute exercise, and in response to eccentric training, is still unclear. Our results support that a myostatin downregulation is important for loading-induced adaptations of skeletal muscle and indicate that the effect of short-term eccentric training on myostatin regulation is greater than the effects of concentric and isometric training. However, it should be considered that changes in mRNA are only indicative of changes in protein levels, and furthermore myostatin action appears to be dependent on enzymatic activation (55). Thus further experiments investigating protein levels of both active and inactive myostatin, as well as IGF-I protein, should be performed to confirm the findings of the present study.

In tendon tissue, we were able to detect myostatin mRNA, but the concentration was highly variable, and no changes were seen in response to training. Thus we found no indications that myostatin regulation is important for tendon adaptation. In contrast, our results indicate that both IGF-IEa and MGF are possible players in adaptation of tendon tissue to increased loading. As shown earlier (43), we found mRNA for both IGF-I splice variants to be present in tendon and furthermore that the concentrations of these mRNA species were similar in tendon and muscle tissue. Interestingly, we saw that their expression increased in response to all types of training (Figs. 3B and 4B). This corresponds well with our earlier observations of increased IGF-IEa and MGF expression in female rat plantaris tendon after functional loading (43). IGF-IEa is known to induce collagen synthesis in rabbit tendon tissue (1, 2), and the observed increase in IGF-IEa mRNA points to this growth factor as a mediator of the increased collagen synthesis seen in human (male and female) tendon in response to acute exercise (32, 38, 39). The effect of MGF on collagen regulation, and on tendon tissue turnover in general, has not been investigated. However, our observations indicate a likely involvement of this growth factor in tendon adaptation, and the function of MGF in tendon tissue remains an attractive area for future investigation.

The roles of IGF-IEa and MGF in skeletal muscle are extensively described, and both splice variants are known to be involved in loading-induced hypertrophy (16). In line with earlier studies that show increased IGF-I mRNA levels in the early phase of muscle loading (4, 7, 10, 11), we found increased muscle levels of IGF-IEa and MGF mRNA after 4 days of resistance training. Recent studies have shown a discrepancy in the timing of MGF and IGF-IEa expression in response to mechanical loading in rat muscle (17, 24). Haddad and Adams (17) found a more rapid increase in MGF expression compared

with that of IGF-IEa in response to stimulated isometric contractions in female rat muscle, and a similar picture was shown in response to stretch combined with stimulation by Hill and Goldspink (24). These findings suggest that after muscle loading the IGF-I precursor mRNA is first spliced toward the MGF variant and then later toward the IGF-IEa variant (24). In the present study, the fold increases in MGF mRNA appeared higher than the increases in IGF-IEa mRNA in both muscle and tendon tissue (Figs. 3 and 4), indicating a relatively greater increase in MGF expression compared with IGF-IEa.

With regard to the specific effect of contraction type, we found eccentric training to have greater effect on muscle IGF-IEa and MGF mRNA expression than concentric training. In addition, the effect of isometric training on IGF-IEa mRNA was superior to concentric training. To our knowledge only two studies have been published that compare the effects of pure eccentric loading to other loading types on expression of IGF-I in muscle tissue. Bamman et al. (7) measured muscle IGF-I mRNA 48 h after a single bout of eccentric or concentric exercise in young men and women and found an increase only in response to eccentric exercise. In conflict with this, Adams et al. (3) observed no effect of long-term eccentric training on levels of IGF-IEa or MGF in female rat muscle, while increases were found in response to both concentric and isometric training. The different nature of the muscle stimuli applied in these studies complicates the comparison, and it should be noted that, conversely to the present study, no significant differences between contraction types were reported in either study (3, 7). Our results could be expected to concur with the findings of Adams et al. (3), as the set-up for training was similar. Meanwhile, we applied four consecutive days of training and a considerably higher workload per session, and considering the known effect of repeated eccentric contractions (46), the possibility of muscle damage induced by eccentric training was presumably greater in the present study compared with the work by Adams et al. (3). Muscle damage has been shown to increase local IGF-I protein levels in rats and humans (22, 51, 57), and if muscle damage was induced in response to eccentric loading with our training approach, and not with the training applied in the study of Adams et al., this might explain the discrepancy with regard to the expression of IGF-IEa and MGF. However, if the increase in IGF-IEa and MGF in response to eccentric training were based primarily on muscle damage, and a possible inflammatory response, eccentric loading would be expected to have a greater effect than isometric loading, and although the fold changes seen after eccentric loading appeared higher than after isometric loading, no significant difference was observed between these training types. Thus no conclusive explanations can be given for the diverging observations on the effect of eccentric exercise on IGF-I regulation.

When taking into account the changes we found in myostatin, IGF-IEa, and MGF, and considering the contrasting effects of myostatin and the IGF-I splice variants on muscle growth, eccentric training does appear to have greater potential for inducing muscle growth than other training types. With an identical set-up for training as the one employed in the present study, Adams et al. (3) showed a torque production during eccentric contraction, which was approximately 1.9- and 3-fold higher than during isometric and concentric contraction, although the stimulation parameters were constant. Thus, as

discussed in several earlier studies (e.g., Refs. 21, 23), the obvious explanation for the superior effect of eccentric loading is the higher total workload imposed on the muscle. This could lead to a greater response, not only locally in the eccentrically loaded muscle tissue, but also in the central hormones (e.g., growth hormone) (29), which could in turn affect skeletal muscle expression of growth factors (19). In support of this, we found the extent of mRNA changes to be ranked in an order corresponding to the level of torque production (eccentric > isometric > concentric), although differences between eccentric and isometric training were not significant for IGF-IEa and MGF (Figs. 2A, 3A, and 4A). Meanwhile our results on tendon tissue add an interesting dimension to this discussion, as we saw no differences in the induction of IGF-IEa and MGF mRNA between contraction types in this tissue. This indicates that muscle tissue is either more sensitive than tendon to changes in workload or that it contains a sensing mechanism that enables it to detect differences between the specific contraction modes, perhaps involving detection of lateral forces or shear stress. In support of this, data reported by Moore et al. (41) indicate a higher myofibrillar protein synthesis rate in young men in response to acute lengthening contractions compared with shortening contractions, even though workloads were identical.

**Limitations of the study.** In the present study we chose to investigate only pure contraction modes. Earlier work indicates that a combination of concentric and eccentric action may be the optimal training type in relation to hypertrophy, strength gain, and hormonal response (13, 21, 29), and such a combination of concentric and eccentric muscle action may elicit an even greater response than the one seen in response to eccentric loading. Thus, in future studies it would be relevant to include a training type, which combines concentric and eccentric muscle action. Furthermore, it should be kept in mind that the training employed in the present study was based on involuntary muscle contractions, and we cannot be certain that the effect of this type of training is identical to that of a voluntary type (28). Finally, it should be considered that female rats were investigated, and because of differences in hormonal response between genders (30), the observed responses may not have been identical in male rats.

In conclusion, we have demonstrated that short-term training increases tendon levels of both IGF-IEa and MGF mRNA, indicating a possible role for these growth factors in the adaptation of tendon to training. Furthermore, we found that eccentric training was more effective in downregulating myostatin expression than other loading types, and in combination with the effect of eccentric loading on IGF-IEa and MGF expression, this may well explain the strong contributions of eccentric actions in resistance training-induced muscle hypertrophy.

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