Systemic IGF-I administration attenuates the inhibitory effect of chronic arthritis on gastrocnemius mass and decreases atrogin-1 and IGFBP-3

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López-Menduiña M, Martín AI, Castillero E, Villanúa MA, López-Calderón A. Systemic IGF-I administration attenuates the inhibitory effect of chronic arthritis on gastrocnemius mass and decreases atrogin-1 and IGFBP-3. Am J Physiol Regul Integr Comp Physiol 299: R541–R551, 2010. First published June 2, 2010; doi:10.1152/ajpregu.00211.2010.—Adjuvant arthritis is an animal model of rheumatoid arthritis that decreases liver and circulating IGF-I as well as skeletal muscle mass. The aim of this work was to elucidate whether IGF-I administration was able to prevent the effect of arthritis on body weight and on two skeletal muscles, gastrocnemius and soleus. On day 4 after adjuvant injection, control and arthritic rats were treated with IGF-I (100 μg/kg sc) two times a day, until day 15 when all rats were killed. Arthritis decreased body weight gain and gastrocnemius weight. In arthritic rats, IGF-I treatment increased body weight gain and gastrocnemius weight, without modifying food intake or the external signs of arthritis. Arthritis increased atrogin-1 and muscle ring finger 1 (MuRF1) gene expression in the gastrocnemius and to a lesser extent in the soleus muscle. IGF-I attenuated the arthritis-induced increase in atrogin-1 and MuRF1 expression in the gastrocnemius, whereas it did not modify the expression of these genes in the soleus muscle. Arthritis also increased IGF-binding protein (IGBP)-3 and IGFBP-5 gene expression in gastrocnemius and soleus, whereas IGF-I administration decreased IGFBP-3, but not IGFBP-5, gene expression in both muscles. In both groups of arthritic rats and in control rats treated with IGF-I, proliferating cell nuclear antigen and myogenic differentiation proteins were increased in the gastrocnemius. These data suggest that the inhibitory effect of chronic arthritis on skeletal muscle is higher in fast glycolytic than in slow oxidative muscle and that IGF-I administration attenuates this effect and decreases atrogin-1 and IGFBP-3 gene expression.

adjuvant-induced arthritis; soleus; muscle ring finger 1; MyoD; proliferating cell nuclear antigen

INFLAMMATORY ILLNESSES INDUCE adipose and skeletal muscle wasting leading to cachexia (61). Rheumatoid arthritis is a progressive illness that causes articular cartilage destruction, ankylosis of the joints, and functional disability. Cachexia has been described in patients with rheumatoid arthritis, adversely affecting morbidity and mortality (47). Adjuvant arthritis is an experimental model of rheumatoid arthritis that can be induced in rats by an intradermal injection of Freund’s adjuvant. After injection (10 days), rats develop chronic inflammation, polyarthritis, a decrease in body weight gain, and a loss of muscle mass (9).

It is generally accepted that inflammatory cachexia is a multifactorial process, being the result of an increased release of inflammatory mediators and endocrine modifications (40). The endocrine response to adjuvant-induced arthritis is characterized by an increase in glucocorticoid secretion (53) together with a decrease in secretion of anabolic hormones such as testosterone (11), growth hormone (GH), and IGF-I (24, 53).

A decrease in serum IGF-I levels in patients with chronic arthritis has also been observed (1). Therefore, weight loss during chronic inflammation may result, at least in part, from a lack of circulating IGF-I. Accordingly, we have previously observed a correlation between the decrease in body weight gain and serum IGF-I in arthritic rats (35).

In inflammatory diseases, skeletal muscle wasting is associated with an accelerated breakdown of myofibrillar proteins by the ubiquitin-proteasome proteolytic pathway (44). The key enzymes in this process are two E3 ubiquitin ligases, atrogin-1 and muscle ring finger 1 (MuRF-1), which are sensitive markers of muscular atrophy (4, 31). In arthritic rats, expression of both enzymes is increased in the gastrocnemius muscle (23), suggesting an increased proteolysis through the ubiquitin-proteasome pathway.

The maintenance of skeletal muscle mass is controlled by both systemic and locally produced factors. IGF-I is among the hormonal factors with major actions on muscle that plays an important role in regulating postnatal growth and stimulating skeletal muscle mass (for review, see Ref. 12). Circulating IGF-I decreases under a number of different conditions such as fasting, disease, or inflammation. Furthermore, although short-term exercise increases circulating IGF-I levels, prolonged exercise also decreases this hormone (10). It is difficult to know the effect of circulating IGF-I on muscle growth and regeneration, since IGF-I can act either as a classic circulating hormone derived from the liver or as a local growth factor. Transgenic mice overexpressing IGF-I in the liver show increased circulating IGF-I and muscle mass (33). However, other authors indicate that maintenance of muscle mass is mainly due to IGF-I locally produced in the muscle, since deletion of liver IGF-I gene resulted in a 75% reduction in circulating IGF-I. Regardless of this, these mice had normal growth (59). In several models of cachexia, such as sepsis and cancer, IGF-I expression in the muscle is decreased (13, 15). However, in arthritic rats, IGF-I is not decreased, but it is increased in gastrocnemius muscle (23).

IGF-I actions can be modified by a family of six binding proteins that regulate IGF-I half-life and access to IGF-I receptors (for review, see Ref. 37). Although the IGF-binding proteins (IGFBPs) are expressed by all tissues in the body, their pattern of expression is tissue specific. Skeletal muscle produces several IGFBPs, with IGFBP-3 and -5 being the most abundant (51). IGFBP-3 is the main circulating protein that binds IGF-I, which is thought to increase the half-life of circulating IGF-I and to control access of IGF-I to extravascu-
lar target tissues (37). IGFBP-3 can also act as a local protein that has been reported to inhibit growth and enhance apoptosis in an IGF-dependent as well as in an IGF-independent manner (38). IGFBP-5 is another IGFBP that is predominant in skeletal muscle (29) and whose overexpression causes impaired growth (48). Like IGFBP-3, IGFBP-5 is also able to inhibit growth by an IGF-independent action during development (57). Arthritis also increases muscle IGFBP-3 and IGFBP-5 expression. IGFBP-3 is increased from day 10 after adjuvant injection, before muscle IGF-I and IGFBP-5 are increased (9). For that reason, it is possible that IGFBP-3 and IGFBP-5 prevent muscle IGF-I from stimulating muscle, in spite of it being increased in arthritic rats.

Taking into account that circulating, but not local, IGF-I is decreased in arthritic rats, we hypothesize that exogenous administration to arthritic rats may ameliorate the catabolic state in arthritic rats. The aim of the present study was therefore to elucidate whether exogenous IGF-I was able to modify the inhibitory effect of adjuvant-induced arthritis on muscle and body weight. The effect of treatment with IGF-I was analyzed on two muscles, gastrocnemius as a type of predominantly white (glycolytic) fast-twitch muscle and in soleus as a predominantly red (oxidative) slow-twitch muscle. We have measured atrogin-1 and MuRF1 expression as markers of the ubiquitin-proteasome activity in both muscles. The myogenic regulatory factors involved in proliferation and differentiation of muscular precursor cells, proliferating cell nuclear antigen (PCNA), myogenic differentiation protein (MyoD), and myogenin, were also determined, since they are increased in the gastrocnemius of arthritic rats (9).

MATERIALS AND METHODS

Arthritic and control male Wistar rats were purchased from Charles River (Barcelona, Spain). Arthritis was induced in the rats by a subcutaneous injection of 4 mg heat-inactivated Mycobacterium butyricum in the right hindpaw, under isoflurane anesthesia (9). Control animals were injected with vehicle (0.1 ml of paraffin oil). After arriving (day 3 after adjuvant injection), rats were housed three to four per cage and maintained under standardized conditions of temperature (20–22°C) and light (lights on from 7:30 A.M. to 7:30 P.M.). Assessment of arthritis was performed by measuring the arthritis index of each animal, which was clinically scored by grading each paw from 0 to 4. Grading was determined as: 0, no erythema or swelling; 1, slight erythema or swelling of one or more digits; 2, swelling of paw; 3, swelling of entire paw and ankle; 4, ankylosis, swelling; 1, slight erythema or swelling of one or more digits; 2, swelling of paw from 0 to 4. Grading was determined as: 0, no erythema or swelling of paw; 3, swelling of entire paw and ankle; 4, ankylosis, swelling; 1, slight erythema or swelling of one or more digits; 2, swelling of paw; 3, swelling of entire paw and ankle; 4, ankylosis, swelling.

Experimental protocol. On day 4 after adjuvant injection, both control and arthritic rats were randomly divided into two groups, each with 10 rats. One group was treated with 250 μl of saline subcutaneously two times a day (at 9:00 A.M. and at 7:00 P.M.). The second group received similar treatment with 100 μg/kg body wt of recombinant human IGF-I (Groppep, Adelaida, Australia) dissolved in saline. At this dosage (100 μg/kg every 12 h), IGF-I is able to decrease liver injury induced by ischemia-reperfusion (7). Furthermore, at a lower dosage (20 μg/kg divided into 2 doses), IGF-I induces a significant reduction in oxidative damage in rats with advanced liver cirrhosis (22). Because arthritis decreases food intake, a pair-fed group injected with saline was also included. Pair-fed rats received the same amount of food (g/100 g body wt) eaten on the previous day by arthritic rats treated with saline.

Body weight, food intake, and arthritis index scores were examined daily. All rats were killed by decapitation 15 days after adjuvant injection and after 11 days of IGF-I treatment, 2.5 h after the last injection. Trunk blood was collected in cooled tubes, allowed to clot, and centrifuged, and the serum was stored at −20°C until IGF-I and IGFBP-3 assays were performed. Immediately after decapitation, spleen and visceral (epididymal) and subcutaneous (the interscapular fat pad from the back) white adipose tissues were removed and weighed. Left gastrocnemius and soleus were dissected, frozen in liquid nitrogen, and stored at −80°C until RNA or protein extraction. The left hindpaw was amputated at the ankle, and its volume was measured by water displacement. Isolation and manipulation of tissues were always performed under sterile conditions.

RNA extraction and real time-PCR. Gastrocnemius or soleus muscles (100 mg) were homogenized, and total RNA was extracted using Ultraspec (Biotech Laboratories, Houston, TX), following the manufacturer’s protocol. The final concentration of RNA was determined with a BioPhotometer (Eppendorf, Germany), and the integrity of the RNA was confirmed by agarose gel electrophoresis. First-strand cDNA synthesis was performed using 1 μg of total RNA with the Quantiscript Reverse Transcription kit (Qiagen Combh Hilden, Valencia, CA). Primers for real-time PCR (Table 1) were obtained from Roche (Madrid, Spain) by using the EXIQON Universal Probe Library (IGFBP-3, IGFBP-5, atrogin-1) or from previously published sequences of IGF-I and MuRF-1 (16), 18S (6), and hypoxanthine-guanine phosphoribosyl transferase (HPRT) (38).

Each real-time PCR reaction consisted of 10 ng total RNA equivalents, 1X Takara SYBR Green Premix Ex Taq (Takara BIO, Otsu, Shiga, Japan), and 300 nM forward and reverse primers in a reaction volume of 25 μl. Reactions were carried out on a SmartCycler (Cepheid, Sunnyvale, CA). Parameters included an initial activation of hotStarTaq DNA polymerase at 95°C for 15 s, followed by 40 cycles of denaturation at 94°C for 15 s, annealing at 60°C, and extension at 72°C for 30 s. Specific amplification was confirmed by the presence of one single peak in the melting curve plots. In addition, the PCR products were analyzed by agarose gel electrophoresis. Results were calculated as the percentage of control rats, using the cycle threshold 2 (ΔΔCt) method (34) with HPRT or 18S as the reference genes.

Table 1. Primers for real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer (5’ to 3’)</th>
<th>Reverse Primer (5’ to 3’)</th>
<th>Product, bp</th>
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<tr>
<td>IGS</td>
<td>GCTGCACGTGGCGGTTCTTA</td>
<td>TCGTGCTGTATCCGGAATTAAACCC</td>
<td>60</td>
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<tr>
<td>HPRT</td>
<td>CTCATGAGCAAGTATGAGGACAGGAC</td>
<td>GCAAGCTCAGAAGAACCTATGACCC</td>
<td>122</td>
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<tr>
<td>Atrogin-1</td>
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<td>GCCTCTTACAGCTCTCTTGTGGAA</td>
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<td>MuRF-1</td>
<td>TGTCGCTGCTGGTTTCCG</td>
<td>ATGCGGCTCAGATGCCTTGT</td>
<td>58</td>
</tr>
<tr>
<td>IGF-1</td>
<td>GTCTAGCTGCAAGGATCCG</td>
<td>TCAGGACGACGACATCCG</td>
<td>62</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>GGAAAGACGGAGTGGATTG</td>
<td>GGGTATTGCGACTCCGACGT</td>
<td>78</td>
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<tr>
<td>IGFBP-5</td>
<td>GCGGACCAAGACAGATGAGA</td>
<td>GGCTCCTGACGGCTCTG</td>
<td>75</td>
</tr>
</tbody>
</table>

HPRT, hypoxanthine-guanine phosphoribosyl transferase; MuRF-1, muscle ring finger-1; IGFBP, insulin-like growth factor-I-binding protein.
Immunoblot. Muscle samples (100 mg each) were homogenized in 1 ml lysis buffer with protease inhibitor cocktail (Sigma-Aldrich, Madrid, Spain). The homogenate was later centrifuged at 13,000 rpm at 4°C for 30 min to remove tissue debris. Protein concentration was determined using the Bradford protein assay with BSA as standard. The protein extract was boiled for 5 min with a 1:1 volume of Laemmli loading buffer. Proteins (100 μg) were resolved by electrophoresis on 14% polyacrylamide gels under reducing conditions and then transferred to nitrocellulose membranes that were blocked by incubation in 5% nonfat dry milk, 0.1% Tween (Sigma-Aldrich) in Tris-buffered saline. Membranes were probed overnight at 4°C, sequentially with antibodies against myogenin, PCNA, and MyoD (Santa Cruz Biotechnology, Santa Cruz, CA) and α-tubulin (Sigma-Aldrich) with stripping of membranes before each new antibody. Membranes were then incubated for 90 min in the appropriate secondary antibody conjugated to horseradish peroxidase [anti-mouse IgG (Amersham Biosciences, Little Chalfont, UK); anti-rabbit IgG (Bio-Rad, Madrid, Spain)], and peroxidase activity was detected using enhanced chemiluminescent reagent (Amersham Biosciences). Band intensities were quantified by densitometry using a PC-Image VGA24 program for Windows. The density of the protein band in each lane was expressed as the percentage of the mean density of control rats, after load normalization using α-tubulin.

IGF-I determination. Total IGF-I (human IGF-I + rat IGF-I) was measured as previously described (45), using the antisera to human IGF-I (UB2-495) that was a gift from Dr. L. E. Underwood and Dr. J. J. Van Wik and is distributed by the National Institute of Diabetes and Digestive and Kidney Diseases Hormone Distribution Program through the National Hormone and Pituitary Program. Levels of IGF-I were expressed in terms of human IGF-I from Gropep and correspond to both exogenous injected human IGF-I and endogenous rat IGF-I, since the antisera to human IGF-I shows a cross-reaction with IGF-I from other species, including rat IGF-I. The intra-assay coefficient of variation was 8%. All samples were run in the same assay. Endogenous rat-IGF-I in the serum was measured using a specific RIA for mouse/rat IGF-I. RIA for rat IGF-I was purchased from DRG Instruments (Marburg, Germany); it did not cross-react with human IGF-I and was performed following the manufacturer’s instructions (www.drg.diagnostics.de).

Statistical analysis. Statistics were computed using the statistics program STATGRAPHICS plus for Windows. Data are presented as means ± SE and were tested with ANOVA; post hoc comparisons were made using the least-significant difference multiple-range test. Data that were not normally distributed (atrogin-1 and MuRF1 mRNA) were analyzed by Mann-Whitney (Wilcoxon) W-test. Statistical significance was set at P < 0.05.

RESULTS

The evolution of the arthritis score index, food intake, and body weight gain during the experiment are shown in Fig. 1. During the first phase of the illness, until the 10th day after adjuvant injection, arthritic rats had a small inflammatory reaction in the injected paw, with arthritis scores between two and three (Fig. 1A), but not in the other paws. On day 10, the polyarticular inflammation started, where the arthritis scores reached the maximum value on day 15 in the arthritic rats treated with saline. On day 15 after adjuvant injection, the spleen weight and the left paw volume were higher in the arthritic rats than in control or pair-fed rats (Table 2). IGF-I administration did not modify spleen weight or left paw volume in control or arthritic rats (Table 2).

Arthritis decreased food intake (P < 0.01) on all days studied (Fig. 1B). The lowest food intake, in both groups of arthritic rats, was observed between days 10 and 13, coinciding with the rapid increase in the arthritis index score. IGF-I...
administration did not modify food intake in control or in arthritic rats (Fig. 1B). As expected, body weight gain in the arthritic rats injected with saline was lower than in the control rats from day 5 (P < 0.01; Fig. 1, C and D). This decrease between days 5 and 9, before the polyarticular inflammation started, seems to be due to the anorexigenic effect of arthritis, since arthritic rats showed similar body weight gain to the pair-fed rats (Fig. 1C). However, from day 10 to 15, body weight gain in arthritic rats treated with saline was lower than in pair-fed rats.

IGF-I increased body weight gain in arthritic rats. The few first days of the study (days 6 and 7), arthritic rats injected with IGF-I had even higher body weight gain than pair-fed rats. Arthritic rats treated with IGF-I had higher body weight gain than the rats treated with saline on most of the days studied (Fig. 1C). In contrast, IGF-I administration to control rats did not modify body weight (Fig. 1D).

The decrease in body weight in arthritic rats is concomitant with a decrease in both visceral and subcutaneous white adipose tissue mass (Table 2). This decrease in white adipose tissue can be in part secondary to the lower food intake, since pair-fed rats had lower visceral and subcutaneous white adipose tissue weights than control rats. IGF-I administration to arthritic rats decreased relative visceral white adipose tissue to
values lower than those of pair-fed rats ($P < 0.05$). In control rats, IGF-I administration decreased subcutaneous and visceral white adipose tissue weight, but the decrease was only significant in subcutaneous white adipose tissue (Table 2). In addition to the decrease in white adipose tissue mass, arthritis also induced a significant ($P < 0.01$) decrease in gastrocnemius weight ($P < 0.01$; Fig. 2, A and B), but this decrease was not observed in pair-fed rats when their gastrocnemius weight was normalized to body weight (Fig. 2B). IGF-I administration to arthritic rats increased gastrocnemius weight, whereas it had no effect in control rats. The effect of arthritis on soleus weight was much lower than on gastrocnemius weight. Arthritic rats had lower soleus weight than the control rats (Fig. 2C), but this decrease was not significant when comparing normalized soleus weights (Fig. 2D).

There was a significant decrease in serum concentrations of rat IGF-I both in arthritic and in pair-fed rats, whereas IGF-I administration did not modify the serum concentrations of rat IGF-I in control or in arthritic rats (Fig. 3A). However, IGF-I administration increases serum concentrations of total IGF-I (human + rat), but this increase was only significant in the control rats injected with IGF-I (Fig. 3B).

As shown in Fig. 4, A and B, arthritis increased the expression of both atrogenes atrogin-1 and MuRF1 in the gastrocnemius ($P < 0.01$). IGF-I administration decreased atrogin-1 mRNA in the gastrocnemius, but this decrease was only significant in arthritic rats (Fig. 4A). IGF-I administration also decreased MuRF1 expression in the gastrocnemius of control rats. Arthritis increased gene expression of atrogin-1 and MuRF1 in the soleus (Fig. 4, C and D), but to a lower extent than in the gastrocnemius. IGF-I administration did not modify atrogin-1 or MuRF1 expression in the soleus of control or arthritic rats.

IGF-I gene expression in the gastrocnemius was increased in arthritic rats, and IGF-I administration did not modify IGF-I mRNA in either arthritic or control rats (Fig. 5A). Neither arthritis nor IGF-I administration modified IGF-I expression in the soleus (Fig. 5B). However, food restriction decreased IGF-I gene expression in soleus, since pair-fed rats have lower IGF-I mRNA levels than control or arthritic rats. Arthritis increased IGFBP-3 gene expression in the gastrocnemius and in the soleus, where the increase was lower in the soleus than in the gastrocnemius (Fig. 5, C and D). IGF-I administration decreased IGFBP-3 expression in both muscles, but this decrease was significant only in arthritic rats (Fig. 5E). Arthritis increased IGFBP-5 mRNA in the gastrocnemius to levels higher than those observed in pair-fed rats (Fig. 5E). However, the increase in IGFBP-5 was lower than the increase in IGFBP-3 ($1.4 \pm 0.015$ vs. $5.2 \pm 0.7$, $P < 0.01$). IGFBP-5 mRNA in the soleus of arthritic rats was higher than in control or pair-fed rats (Fig. 5F). IGF-I administration did not modify IGFBP-5 gene expression in muscles of control or arthritic rats.

Figure 6, A–C, shows PCNA, MyoD, and myogenin levels in the gastrocnemius. Arthritis induced a significant increase in the three proteins in the gastrocnemius. In control rats treated with IGF-I, PCNA and MyoD were similar to those observed in arthritic rats, whereas myogenin was not affected. IGF-I administration did not modify these proteins in the gastrocnemius of arthritic rats.

As shown in Fig. 7, the effect of arthritis on the myogenic regulatory factor was lower in the soleus than in the gastrocnemius. Arthritis increased MyoD in the soleus (Fig. 7B), but neither PCNA nor myogenin was significantly modified by arthritis or IGF-I treatment.

**DISCUSSION**

The present data indicate that exogenous IGF-I administration to arthritic rats is able to increase body weight and to ameliorate skeletal muscle wasting. The stimulatory effect of IGF-I on body weight is not secondary to modifications in food intake or in the inflammatory response, since IGF-I administration did not ameliorate anorexia, arthritis scores, paw volume, or the spleen weight in arthritic rats.

The decrease in body weight gain in arthritic rats was, in part, secondary to reduced food intake. However, the lower

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**Fig. 3.** Serum concentrations of rat-IGF-I (A) and total IGF-I (human + rat-IGF-I) (B) in control, AA, or PF rats treated with 100 mg/kg sc of IGF-I 2 times/day or with saline from day 4 to day 15 after adjuvant injection. Serum concentrations of rat and total IGF-I were decreased in AA and PF rats ($P < 0.01$). IGF-I administration increased ($P < 0.05$) serum concentrations of total IGF-I in control rats. Data are expressed as means ± SE ($n = 9–10$). *Different from control-saline. †Different from control-IGF-I.
body weight gain in arthritic rats compared with pair-fed rats indicates the inhibitory effect of inflammation on body weight. As we have previously reported (9, 36), arthritic rats had lower relative fat and skeletal muscle mass, whereas pair-fed rats only had lower relative fat mass. These data suggest that the arthritis-induced decrease in gastrocnemius weight is due to the inflammatory process rather than to anorexia. IGF-I administration partially prevents the effect of arthritis on body weight, since the body weight evolution of the arthritic rats injected with IGF-I had MuRF1 mRNA levels similar to those of pair-fed rats. IGF-I administration decreased MuRF1 mRNA in the rats treated with saline and IGF-I, but the AA rats treated with IGF-I had MuRF1 mRNA levels similar to those of control rats. IGF-I administration decreased MuRF1 mRNA in the gastrocnemius of control rats (P < 0.05). Arthritic rats had lower relative fat and skeletal muscle mass, whereas pair-fed rats only had lower relative fat mass. These data suggest that the arthritis-induced decrease in gastrocnemius weight is due to the inflammatory process rather than to anorexia. IGF-I administration partially prevents the effect of arthritis on body weight, since the body weight evolution of the arthritic rats injected with IGF-I is similar to that of the pair-fed rats. In arthritic rats, IGF-I increased body weight was a result of its effect on skeletal muscle rather than on white adipose tissue, since IGF-I increased gastrocnemius weight but not white adipose tissue weight. However, in control rats, IGF-I administration did not increase body or skeletal muscle weights. These data are in accordance with those showing that the anabolic effect of IGF-I administration is significant in catabolic states rather than in normal states (25). In healthy humans with normal GH-IGF-I function, acute IGF-I administration increases muscle protein synthesis, but chronic IGF-I administration does not result in increased body lean mass (for review, see Ref. 55). On the contrary, IGF-I administration is able to reduce negative nitrogen balance during caloric restriction and increase muscle mass in hypophysectomized animals (58).

Arthritis has a different effect on the two skeletal muscles analyzed, gastrocnemius and soleus, where the decrease in muscular mass is higher in the gastrocnemius than in the soleus. In fact, on day 15 after adjuvant injection, the weight of the gastrocnemius in arthritic rats was almost one-half that of control rats. However, the decrease in the weight of the soleus in arthritic rats was not significant when comparing relative weights. The different response to catabolic stimuli between predominantly glycolytic skeletal muscle (gastrocnemius) and predominantly oxidative skeletal muscle (soleus) has been reported previously in burn-injured rats (18), in chronic heart failure (32), and in septic rats (39, 56). These data suggest that, in cachexia induced by chronic illness, fast glycolytic muscles are more prone to atrophy than slow oxidative muscles, with the exception of disuse, by 17-day bed rest unloading (47) or by denervation (61), which also induces soleus atrophy. Accordingly, arthritis-induced upregulation of the two atrogenes atrogin-1 and MuRF1 is higher in the gastrocnemius than in the soleus. In addition, the effect of arthritis on IGF-I, IGFBP-3, and myogenic regulatory factors is also higher in gastrocnemius than in soleus. Protection of oxidative muscle against muscle wasting in several chronic diseases has been explained by the fact that oxidative muscle has enhanced response of the antioxidant system compared with glycolytic muscle (60).

As we have previously reported (8, 9), arthritis not only increased expression of both atrogenes in the gastrocnemius but also increased the proliferation marker PCNA, the early myogenic regulatory factor MyoD, and myogenin, a late-acting myogenic regulatory factor, as well as local IGF-I.
On the contrary, those effects of arthritis on the regulatory factors involved in myogenesis are clearly lower in the soleus. Therefore, altered IGF-I, PCNA, MyoD, and myogenin expression can be caused by muscle atrophy, and the increase in these proteins may have a protective role. Similar increases in atrogin-1, MuRF1, IGF-I, MyoD, and myogenin have been described in denervation-induced muscle wasting (14, 27, 61). However, in this model of muscle wasting, both soleus and gastrocnemius muscles became atrophic to the same extent, and there was a similar increase in atrogenes and myogenic regulatory factors in both types of muscle (27, 28, 61). All of those data suggest that, in the gastrocnemius, arthritis stimulates both upregulation of atrogenes and activation of local IGF-I as well as the myogenic regulatory muscle factors, whereas the lower atrophic response in the soleus is associated with there being no modifications in local IGF-I gene expression or myogenic regulatory factors.

The effect of IGF-I administration is also more evident in the gastrocnemius than in the soleus. In the gastrocnemius of arthritic rats, IGF-I decreased atrogin-1 and MuRF1, although the effect on MuRF1 expression is lower and only significant in control rats. In contrast, IGF-I did not decrease atrogin-1 or MuRF1 gene expression in the soleus of arthritic rats, as well as in the soleus of control rats. Data are expressed as means ± SE (n = 8–10). *Different from control-saline. †Different from control-IGF-I. ‡Different from arthritic-saline. §Different from PF.
Fig. 6. Proliferating cell nuclear antigen (PCNA, A), myogenic differentiation protein (MyoD, B), and myogenin (C) in the gastrocnemius of control, AA, and PF rats treated with IGF-I (100 μg/kg sc 2 times/day) or saline from day 4 to day 15 after adjuvant injection. PCNA, MyoD, and myogenin were measured by Western blotting, quantified, normalized against α-tubulin, and expressed as a percentage of the control rats. Both groups of AA rats and control rats treated with IGF-I have increased PCNA and MyoD. Myogenin was increased in both groups of AA rats. Data are expressed as means ± SE (n = 8–10 rats). *Different from control-saline. #Different from control-IGF-I. $Different from PF.

Fig. 7. PCNA (A), MyoD (B), and myogenin (C) in the soleus of control, AA, and PF rats treated with human (h) IGF-I (100 μg/kg sc 2 times/day) or saline from day 4 to day 15 after adjuvant injection. PCNA, MyoD, and myogenin were measured by Western blotting, quantified, normalized against α-tubulin, and expressed as a percentage of the control rats. Arthritis increased MyoD levels. Data are expressed as means ± SE (n = 6–9 rats). *Different from control-saline. #Different from control-IGF-I. $Different from PF.
It has been suggested that locally synthesized IGF-I is more important than circulating IGF-I in the maintenance of muscle mass (58). However, arthritic rats have increased IGF-I gene expression in the gastrocnemius but not in the soleus muscle, whereas muscle atrophy is more evident in the gastrocnemius than in the soleus. One possible explanation can be due to the increase in local IGFBP-3, which is higher in the gastrocnemius than in the soleus and may prevent IGF-I action. It is also possible that increased IGF-I gene expression, together with myogenic stimulatory factors, PCNA, MyoD, and myogenin, in the gastrocnemius of arthritic rats is a local response to muscle atrophy. In this sense, it has been reported that muscle injury increases local IGF-I synthesis, stimulating both cell proliferation and differentiation (17). This second possibility explains the lack of significant modifications to these factors in the soleus muscle, since the deleterious effect of arthritis in the soleus is lower than in the gastrocnemius.

Partial correction of circulating IGF-I was effective in stimulating body weight gain and gastrocnemius mass in our model of arthritic rats. These data indicate that circulating IGF-I plays a role in maintaining muscle mass. However, correction of circulating IGF-I levels by exogenous IGF-I administration did not ameliorate muscle atrophy induced by ANG II administration (5). In contrast, in this model, muscle-specific overexpression of IGF-I blocks muscle wasting (52). The beneficial effect of local IGF-I overexpression indicates that ANG II-induced muscle wasting is related to local IGF-I. It is important to point out that, in this model of cachexia, the IGF-I-IGFBP system in the gastrocnemius is modified in the opposite way as in arthritic rats, since ANG II administration decreases IGF-I and IGFBP-3 gene expression in the gastrocnemius muscle (5). Another wasting condition that decreases IGF-I in the muscle is sepsis (41). As in the case of ANG II administration, in this model, local administration of IGF-I prevents sepsis-induced muscle atrophy (41). On the contrary, neither systemic IGF-I administration (19) nor incubation of muscles from septic rats with IGF-I is able to prevent muscle proteolysis (26).

Similar to our data in arthritic rats, IGF-I administration is able to block the catabolic response in skeletal muscle after burn injury (18). In addition, “in vitro” incubation of muscles from burned rats with IGF-I is also able to decrease muscle proteolysis (20, 21). From all of these data, we can hypothesize that systemic IGF-I administration is able to ameliorate muscle wasting in states with low circulating IGF-I with normal or increased muscular IGF-I.

As we have previously reported, arthritis increased IGFBP-3 expression in the gastrocnemius (9). IGFBP-3 was also increased in the soleus of arthritic rats, but this increase was lower than that observed in the gastrocnemius. An increase in muscle IGFBP-3 has also been reported 2 days after muscle injury, during the early phase of regeneration when muscle is invaded by inflammatory cells, especially by macrophages (30). These authors found that IGFBP-3 is mainly expressed in macrophages adjacent to the injured muscle (30). Overexpression of IGFBP-3, in addition to inhibiting IGF-I binding to its receptor, has IGF-I-independent effects decreasing cell proliferation “per se” (42). Furthermore, IGFBPs inhibition, with an IGF-I haptamer, enhanced muscle fiber regeneration and increased the rate of functional recovery of fast twitch after myotoxic damage (50). We can speculate that the decrease in IGFBP-3 in muscle after IGF-I administration can contribute to the anabolic effect of IGF-I. As we have previously reported (9), arthritis induces a small increase in IGFBP-5 expression in both types of muscles, but IGF-I administration did not modify this protein.

The inhibitory effect of IGF-I on muscle IGFBP-3 contrasts with previous data reported in the liver (5). Regulation of IGF-I and its binding proteins seems to be different in the liver than in the skeletal muscle. In fact, GH administration increases circulating IGF-I, whereas it does not modify skeletal muscle IGF-I mRNA (2). Furthermore, systemic IGF-I administration increases circulating IGFBP-3 but not IGFBP-3 mRNA in the gastrocnemius muscle (5). Similarly, IGF-I synthesis in the liver is different than in other tissues. For example, administration of clenbuterol, a β2-adrenoceptor agonist, increases IGF-I and IGFBP-5 gene expression in the soleus, but it decreases circulating IGF-I (3).

In conclusion, our data suggest that arthritis-induced muscle wasting can be partly attenuated by exogenous IGF-I. In addition to the inhibitory effect of IGF-I on atrogin-1 and MuRF1, the decrease in IGFBP-3 and the stimulatory effect on myogenic regulatory factors in the rats treated with IGF-I can also contribute to the effect of IGF-I in muscle.

Perspectives and Significance

Chronic inflammatory illnesses are associated with skeletal muscle wasting and cachexia, which increase morbidity and mortality. Adjuvant-induced arthritis is an experimental model of rheumatoid arthritis that is associated with an inhibition of the GH-IGF-I system and muscle proteolysis by activation of the ubiquitin-proteasome pathway. In this study, IGF-I was given systemically to arthritic rats to analyze the role of circulating IGF-I on muscle mass. We found that IGF-I administration ameliorates, but does not prevent, muscle atrophy and reduces atrogin-1 and IGFBP-3 expression. These data suggest that circulating IGF-I plays an important role in the maintenance of muscle mass and that its decrease during inflammation is one of the causes of cachexia. More studies are also needed to explore regulation of IGFBP-3 in skeletal muscle by GH and IGF-I and to determine their relationship with atrogenes and myogenic regulatory factors during inflammation.

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DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES

2. Aperghis M, Velloso CP, Hamed M, Brothwood T, Bradley L, Bouloux PM, Harridge SD, Goldspink G. Serum IGF-I levels and IGF-I...
IGF-I REDUCES ARTHRITIS-INDUCED MUSCLE WASTING


25. Peinequín A, Mourié C, Birot O, Alonso A, Mathieu J, Clarencon D, Agay D, Chancerelle Y, Multon E. Rat pro-inflammatory cytokine, and...


