Ghrelin receptor agonist GHRP-2 prevents arthritis-induced increase in E3 ubiquitin-ligating enzymes MuRF1 and MAFbx gene expression in skeletal muscle

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Chronic arthritis is a catabolic state associated with an inhibition of the IGF system and a decrease in body weight. Cachexia and muscular wasting is secondary to protein degradation by the ubiquitin-proteasome pathway. The aim of this work was to analyze the effect of adjuvant-induced arthritis on the muscle-specific ubiquitin ligases muscle ring finger 1 (MuRF1) and muscle atrophy F-box (MAFbx) as well as on IGF-I and IGF-binding protein-5 (IGFBP-5) gene expression in the skeletal muscle. We also studied whether the synthetic ghrelin receptor agonist, growth hormone releasing peptide-2 (GHRP-2), was able to prevent arthritis-induced changes in the skeletal muscle. Arthritis induced an increase in MuRF1, MAFbx (P < 0.01), and tumor necrosis factor (TNF)-α mRNA (P < 0.05) in the skeletal muscle. Arthritis decreased the serum IGF-I and its gene expression in the liver (P < 0.01), whereas it increased IGF-I and IGFBP-5 gene expression in the skeletal muscle (P < 0.01). Administration of GHRP-2 for 8 days prevented the arthritis-induced increase in muscular MuRF1, MAFbx, and TNF-α gene expression. GHRP-2 treatment increased the serum concentrations of IGF-I and the IGF-I mRNA in the liver and in the cardiac muscle and decreased muscular IGFBP-5 mRNA both in control and in arthritic rats (P < 0.05). GHRP-2 treatment increased muscular IGF-I mRNA in control rats (P < 0.01), but it did not modify the muscular IGF-I gene expression in arthritic rats. These data indicate that arthritis induces an increase in the activity of the ubiquitin-proteasome proteolytic pathway that is prevented by GHRP-2 administration. The parallel changes in muscular IGFBP-5 and TNF-α gene expression with the ubiquitin ligases suggest that they can participate in skeletal muscle alterations during chronic arthritis.

increase in the risk of many alterations, such as inadequate gas exchange or vein thrombosis in lower limbs. Accordingly, it has been postulated that rheumatoid cachexia is an important contributor in increasing morbidity and premature mortality in rheumatoid arthritis patients (59).

Skeletal muscle atrophy is a debilitating response that occurs in many chronic diseases, such as cancer, sepsis, heart or renal failure, and diabetes. Although adjuvant arthritis decreases food intake, cachexia is not the result of the decrease in caloric intake (48). Chronic arthritis decreases serum concentrations of IGF-I and its mRNA in the liver (37). This decrease is not secondary to modifications in food intake, since pair-fed rats have normal serum IGF-I and IGF-I gene expression in the liver (38). In addition, growth hormone (GH) administration to arthritic rats results in an increase in body weight gain along with an increase in IGF-I gene expression in the liver without modifying food intake (28, 39). These data suggest that the weight loss during chronic inflammation may result, at least in part, from disturbances in anabolic hormones such as IGF-I and GH.

The effect of IGF-I on cellular growth and differentiation can be exerted through endocrine or paracrine/autocrine pathways. In chronic heart failure-induced cachexia, the disturbances in skeletal muscle are associated with a decrease in local expression of IGF-I, whereas serum concentrations of IGF-I are not modified (50). The actions of IGF-I are modulated locally by the IGF-binding protein IGFBPs. A downregulation of IGFBP-5 has been reported by microarray studies in several models of muscular wasting (33). However, skeletal muscle hypertrophy induced by overloading is associated with an increase in IGF-I and with a decrease in IGFBP-5 gene expression, whereas unloading is associated with muscular atrophy and an increase in IGFBP-5 expression (2). These data indicate that modifications in local IGF-I and/or IGFBP-5 may be one of the mechanisms of skeletal muscle dysfunction.

Regardless of the illness, skeletal muscle atrophy is associated with an increase in protein degradation (for review, see Ref. 21). Most of the proteolysis in cachexia-induced skeletal muscle atrophy seems to be because of activation of the ATP-dependent ubiquitin-proteasome proteolytic pathway (30). Ubiquitin is a short peptide that can be conjugated to specific substrates. Conjugation of ubiquitin to proteins occurs in a series of steps involving several enzymes, E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme, and E3

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ubiquitin-ligating enzyme. The key enzymes in this process are E3 ubiquitin ligases, and they confer substrate specificity. Among these, two are increased during skeletal muscle atrophy

- muscle ring finger 1 (MuRF1) and muscle atrophy F-box (MAFbx) or Atrogin-1 (5, 23).

The gene expressions of these E3 ubiquitin-ligating enzymes are the most sensitive markers for muscular atrophy. Taking into account that administration of an inhibitor of the proteasome activity has an anti-inflammatory effect in arthritic rats (42), it is possible that in arthritic rats the ubiquitin-proteasome proteolytic pathway is upregulated as a result of inflammatory stimuli.

Ghrelin, a 28-amino acid endocrine peptide, is mainly secreted by the stomach and has been identified as the endogenous ligand of the GH secretagogues receptor. Ghrelin has a potent GH-releasing effect but stimulates food intake and promotes adiposity by a GH-independent action (53). We have recently reported that, during the active phase of arthritis, administration of growth hormone-releasing peptide-2 (GHRP-2), a synthetic ghrelin receptor agonist, reduced the symptoms of arthritis and the serum concentration of IL-6 (24). The anti-inflammatory effect of ghrelin has also been reported in several models of cachexia, such as chronic heart failure peripheral administration of ghrelin attenuated body weight loss in several models of cachexia, such as chronic heart failure peripheral administration of ghrelin attenuated body weight loss in several models of cachexia, such as chronic heart failure.

Ghrelin is an acid gastric hormone, which is mainly produced in the stomach and has been identified as the endogenous ligand of the GH secretagogues receptor. In addition, ghrelin has been suggested as a treatment to prevent the inflammatory effect, since the paw volume and the arthritis score were lower in the arthritic rats injected with GHRP-2 than in the arthritic rats injected with saline (24).

All rats were killed by decapitation 22 days after adjuvant or vehicle injection and after 8 days of GHRP-2 treatment. 2.5 h after the last injection in a separated room, within 30 s after being removed from their cages. Trunk blood was collected in cooled tubes, and the serum was stored at $-20^\circ C$ until IGF-I analysis was performed. Immediately after decapitation, the liver, heart, and gastrocnemius muscle were removed and stored at $-80^\circ C$.

**RNA extraction.** RNA was extracted by the guanidine thiocyanate method using a commercial kit (Ultraspex RNA; Biotex Laboratories, Houston, TX). The integrity and the concentration of the RNA were confirmed using agarose gel electrophoresis.

**Northern blot analysis.** RNA (20 μg) extracted from the skeletal and cardiac muscles and 30 μg from the liver were separated by formaldehyde-agarose gel electrophoresis and transferred to nylon membranes (Roche Molecular Biochemicals, Barcelona, Spain). Prehybridization was performed for 30 min at 68°C in ULTRAhyb buffer (Ambion, Austin, TX) followed by hybridization for 16 h at the same temperature with IGF-I- and GHR-labeled riboprobes. To verify loading, control hybridization was performed with a 28S DNA probe labeled with [32P]deoxycytidine triphosphate by random primer.

Autoradiographs were analyzed by densitometric scanning using a Gengenius (Syngene, Cambridge, UK). The rat IGF-I gene gives different IGF-I mRNA transcripts that can be visualized by Northern blot analysis and consist of a group of transcripts ranging from 7.5 to 9.0 kbp. Because all these transcripts may potentially be translated to IGF-I, the densitometric results correspond to the sum of the IGF-I transcripts 0.9, 1.7, and 7.5 kbp. The rat GHR gene encodes the GHR and the GHBP mRNA of 4.5 and 1.2 kb; both transcripts were quantified by densitometric analysis, and results refer to the total GHR mRNA. The intensities of autoradiogram signal levels were normalized for 28S ribosomal RNA levels.

**Real-time PCR.** For RT-PCR analysis, 10 μg of cardiac or skeletal muscle total RNA were reverse transcribed in a total volume of 30 μl at 37°C for 60 h with 125 units of Moloney murine leukemia virus RT (Maxim Bioch, San Francisco, CA). Each RT-PCR reaction consisted of 312 ng total RNA equivalents, 1× QuantiTect SYBR Green Master Mix (Qiagen, Valencia, CA), and 300 nM forward and reverse primers in a reaction volume of 25 μl. Reactions were carried out on a SmartCycler (Cepheid, Sunnyvale, CA). Primers for PCR (Table 1) were obtained from previously published sequences tumor necrosis factor-α (TNF-α) and MuRF1 (14) r18S (3) or by using the rat GenBank and the EPIXQON ProbeLibrary IGFBP-5 and MAFbx. Primers were designed to span a single sequence derived from two exons (i.e., separated by an intron in genomic DNA and primary RNA

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Product, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>MuRF1</td>
<td>5′-CTGTCGAGAGGCTTTTGCG-3′</td>
<td>5′-ATGCGGCTTCCATGACTCTT-3′</td>
<td>58</td>
</tr>
<tr>
<td>MAFbx</td>
<td>5′-GAACAGCAAAAACCACAACTGAGTA-3′</td>
<td>5′-GCTGGTATGACTCCCTTGTTGGA-3′</td>
<td>74</td>
</tr>
<tr>
<td>IGFBP-5</td>
<td>5′-GGCGAGCAAGGCAACAGATAGA-3′</td>
<td>5′-GCTGGTATGACTCCCTTGTTGGA-3′</td>
<td>75</td>
</tr>
<tr>
<td>TNF-α</td>
<td>5′-GCCACCCAGGTGCTTGCTG-3′</td>
<td>5′-GCTGGTATGACTCCCTTGTTGGA-3′</td>
<td>100</td>
</tr>
<tr>
<td>r18S</td>
<td>5′-AGGGGAGGGGCAAGCATAGAAGA-3′</td>
<td>5′-CAAGAACACAGAGCTGGCTG-3′</td>
<td>126</td>
</tr>
</tbody>
</table>

MuRF1, muscle ring finger 1; MAFbx, muscle atrophy F-box; IGFBP-5, IGF-binding protein-5; TNF-α, tumor necrosis factor-α.
transcripts to minimize amplification). Parameters included an initial activation of hotStar Taq DNA polymerase at 95°C for 15 min, followed by 40 cycles of denaturation at 94°C for 15 s, annealing at 53°C (MuRF1), 57°C (TNF-α), 52°C (MAFbx), 53°C (IGFBP-5), and 60°C (r18S) for 30 s, 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 in agarose gel electrophoresis. Results were calculated as percentage of control rats injected with saline, using the ΔΔCt method (36) with r18S as the control gene.

RRA. IGF-I concentrations were measured by a double-antibody RIA using the antibody National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) UB2–495 distributed by the Hormone Distribution Program of NIDDK through the National Hormone and Pituitary Program, which was a gift from Drs. L. Underwood and J. Van Wyk. Levels of IGF-I were expressed in terms of IGF-I from Pituitary Program, which was a gift from Drs. L. Underwood and J. Kidney Diseases (NIDDK) UB2–495 distributed by the Hormone Laboratory.

Van Wyk. Levels of IGF-I were expressed in terms of IGF-I from Pituitary Program, which was a gift from Drs. L. Underwood and J. Kidney Diseases (NIDDK) UB2–495 distributed by the Hormone Laboratory.

Pierce, Rockford, IL.

Statistical analysis. Statistics were computed using the statistics program STATGRAPHICS plus for Windows. Statistical significance was calculated by multifactorial ANOVA with arthritis and GHRP-2 administration as factors. Post hoc comparisons were made by using the unpaired Student’s t-test. Correlation between different variables was calculated by linear regression. P values <0.05 were considered significant.

RESULTS

As shown in Table 2, arthritis induced a marked decrease in gastrocnemius weight (P < 0.01) and also decreased the protein concentration in this muscle (P < 0.05), whereas GHRP-2 administration to arthritic rats increased the weight and protein concentration of this skeletal muscle (P < 0.05).

Arthritis induced a significant increase in the two ubiquitin ligases MAFbx and MuRF1 gene expression in the skeletal muscle (P < 0.01; Fig. 1), and GHRP-2 administration to arthritic rats prevented the effect of arthritis on both mRNAs. There was also a significant increase (P < 0.05) in TNF-α mRNA in the skeletal muscle in the arthritic rats injected with saline, whereas GHRP-2 administration reverted the effect of arthritis on TNF-α mRNA levels in the skeletal muscle, reaching levels similar to those observed in control rats (Fig. 1).

As shown in Fig. 2, arthritis decreased the serum concentrations of IGF-I (P < 0.01), and GHRP-2 administration increased the serum concentrations of IGF-I both in control (P < 0.05) and in arthritic (P < 0.05) rats. Serum concentration of GH was not significantly modified by arthritis or by GHRP-2 administration (data not shown). Arthritis decreased the IGF-I mRNA in the liver (P < 0.01; Fig. 2), and GHRP-2 administration increased the IGF-I gene expression in the liver in control and in arthritic rats (P < 0.05). There was a positive correlation between the serum concentrations of IGF-I and the liver IGF-I mRNA (r = 0.68, P < 0.01), whereas there was no correlation between the serum concentrations of IGF-I and the IGF-I mRNA in cardiac or in skeletal muscle. The GHR mRNA in the liver was not modified by arthritis or by GHRP-2 treatment (data not shown). Both arthritis and GHRP-2 administration modified the protein content in the liver (Table 2).

Arthritis increased the protein concentration in the liver (P < 0.01), whereas GHRP-2 administration decreased it (P < 0.01).

The effects of arthritis and GHRP-2 on IGF-I mRNA in the cardiac muscle are shown in Fig. 3. Arthritis did not modify the IGF-I expression in the cardiac muscle. In contrast, GHRP-2 administration increased (P < 0.05) the IGF-I mRNA in the cardiac muscle, but this increase was not significant when the individual means were analyzed separately. Neither arthritis nor GHRP-2 administration modified the GHR or MuRF1 gene expression in the cardiac muscle (data not shown).

The effect of arthritis on IGF-I mRNA in skeletal muscle was opposite to that in the liver, since the IGF-I mRNA in the skeletal muscle was higher in arthritic than in control rats (P < 0.01; Fig. 4). GHRP-2 administration increased the IGF-I mRNA in skeletal muscle in control rats (P < 0.01). Neither arthritis nor GHRP-2 treatment induced significant modifications in GHR gene expression in the skeletal muscle (Fig. 4). Arthritis induced an increase in IGFBP-5 mRNA (P < 0.01) in the skeletal muscle, whereas GHRP-2 administration decreased the IGFBP-5 mRNA both in control (P < 0.05) and in arthritic (P < 0.05; Fig. 4) rats.

DISCUSSION

In the present study, chronic arthritis resulted in an increase in gene expression of the ubiquitin ligases E3, MuRF1, and MAFbx as well as in TNF-α and IGFBP-5 in the skeletal muscle, and these upregulations were blocked by treatment with the ghrelin analog GHRP-2. These findings suggest that, in addition to its anti-inflammatory effect in arthritic rats, the synthetic ghrelin analog is also able to decrease arthritis-induced muscle proteolysis.

Chronic arthritis induced a decrease in the weight and protein concentration of the skeletal muscle along with an increase in the expression of ubiquitin ligases MuRF1 and MAFbx.

Table 2. Effect of arthritis and 8-day administration of GHRP-2 on gastrocnemius weight and protein concentration in gastrocnemius, heart, and liver

<table>
<thead>
<tr>
<th></th>
<th>Control-Saline (n = 10)</th>
<th>Control-GHRP-2 (n = 10)</th>
<th>AA-Saline (n = 11)</th>
<th>AA-GHRP-2 (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrocnemius, mg</td>
<td>1.870 ± 0.45</td>
<td>1.931 ± 0.27</td>
<td>593 ± 26*</td>
<td>669 ± 23†</td>
</tr>
<tr>
<td>Gastrocnemius protein, μg/100 mg</td>
<td>4.34 ± 0.23</td>
<td>4.86 ± 0.19</td>
<td>3.75 ± 0.2†</td>
<td>4.36 ± 0.21†</td>
</tr>
<tr>
<td>Heart protein, μg/100 mg</td>
<td>4.33 ± 0.5</td>
<td>4.25 ± 0.25</td>
<td>4.02 ± 0.26</td>
<td>4.06 ± 0.46</td>
</tr>
<tr>
<td>Liver protein, μg/100 mg</td>
<td>9.0 ± 0.2</td>
<td>7.9 ± 0.2*</td>
<td>10.4 ± 0.3*</td>
<td>9.3 ± 0.5*</td>
</tr>
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</table>

Data are means ± SE; n, no. of rats. GHRP-2, growth hormone-releasing peptide-2; AA, arthritic. Two-way ANOVA revealed that arthritis decreased the weight [F(1,39) = 1837, P < 0.01] and the protein concentration [F(1,38) = 4.6, P < 0.05] of the gastrocnemius, whereas it increased [F(1,39) = 19, P < 0.01] the liver concentration of proteins. GHRP-2 administration increased the gastrocnemius weight [F(1,39) = 5.4, P < 0.05] and its protein concentration [F(1,38) = 4.8, P < 0.05], but decreased the protein concentration in the liver [F(1,39) = 12, P < 0.01]. Two-way ANOVA: *P < 0.01 and †P < 0.05 vs. control-saline. ‡P < 0.05 vs. AA-saline.
The role of IGF-I in muscle atrophy is not well known. A decrease in IGF-I mRNA in the skeletal muscle has been detected in muscular wasting induced by hindlimb suspension (2), sepsis (14), and chronic heart failure (50). However, an increase has also been reported in muscular atrophy induced by denervation, chronic human disuse, or chronic skeletal muscle ischemia in humans (9, 45, 55). In addition, no changes in IGF-I expression in the skeletal muscle were detected by microarray analysis in skeletal muscle atrophy induced by cancer, diabetes, or uremia (33). Furthermore, IGF-I overexpression in transgenic mice did not prevent muscle atrophy induced by hindlimb unloading (12). It is possible that the increased muscular IGFBP-5 binds IGF-I and inhibits IGF-I action by preventing its interaction with the type I receptor, as it has been previously reported (31).

Different IGFBP-5 effects on cell proliferation have been reported depending on the tissue and the circumstances. Transgenic mice overexpressing IGFBP-5 are growth retarded and have a decreased skeletal muscle weight relative to body weight (49). Moreover, local application of IGFBP-5 to paralyzed muscle prevents the stimulation of interstitial cell proliferation (9). In contrast, antiapoptotic stimulation of cell survival effects of IGFBP-5 have also been reported in myoblasts and chondrocytes (10, 32). Both an increase and a decrease in muscular IGFBP-5 mRNA in muscular atrophy have been reported (23). Similarly to our data, in chondrocytes isolated from osteoarthritic cartilage, IGF-I and IGFBP-5 gene expressions are increased (41). In arthritis, the increase in IGFBP-5 can be secondary to the increase in cytokine release, since IL-1 and TNF-α are able to stimulate IGFBP-5 in articular chondrocytes (51).

Cytokines also modulate the activity of the ubiquitin pathways in situations such as cancer and sepsis (1). Taking into account that TNF-α activates nuclear factor-κB, and this activation promotes muscle atrophy through MuRF1 upregulation (8), the increased MuRF1 and MAFbx gene expressions in the...
arthritic rats can be because of the marked increase in TNF-α gene expression in the skeletal muscle. The gene expression of the proinflammatory cytokine TNF-α in the skeletal muscle increases in several skeletal muscle alterations, such as diabetes, sepsis, and chronic heart failure (14, 15, 50). It has been postulated that TNF-α exerts its catabolic effects in skeletal muscle in a paracrine/autocrine manner (20). TNF-α influences both muscle protein synthesis and degradation (58). TNF-α blockade prevents muscle protein breakdown by suppressing the activation of the ubiquitin-proteasome-dependent proteolysis in cancer and septic rats (11, 37, 58).

A possible cause of the IGF-I resistance in the muscle of arthritic rats, in addition to IGFBP-5, can be the increase in TNF-α gene expression in the skeletal muscle. TNF-α is able to impair IGF-I-induced protein synthesis in myoblasts. This is not done at the level of receptor but rather by targeting some signaling proteins associated with the IGF-I receptor (6). IGF-I resistance in the muscle at the postreceptor level has also been described in septic rats, in which IGF-I is not able to reduce muscle proteolysis in vivo or in vitro (19, 27).

The IGF-I response to GHRP-2 is small and more evident in control rats than in arthritic rats. Similarly, ghrelin administration two times a day for 5 days was unable to increase serum concentrations of GH or IGF-I in control or in LPS-injected rats (26). After GHRP-2 administration (1 h), no significant increases in serum concentration of IGF-I or insulin have been detected in normal mice (54). These data can be explained by the fact that the stimulatory effect of ghrelin or ghrelin receptor

Fig. 2. Serum concentrations of IGF-I (A) and IGF-I mRNA in the liver (B) in control or arthritic rats injected with saline or 100 μg/kg GHRP-2 for 8 days. D: representative Northern blot of IGF-I mRNA hybridization of total liver RNA. The size of the hybridization band (in kb) is indicated on left: 28S, 28S ribosomal RNA; C, control, S, saline; G, GHRP. Quantitative analyses are expressed as percentages of control rats injected with saline. 28S mRNA expression was used as a control for mRNA. Arthritis decreased the serum concentrations of IGF-I [F(1,35) = 37, P < 0.01] and the IGF-I mRNA in the liver [F(1,37) = 16.8, P < 0.01]. GHRP-2 administration increased IGF-I levels in serum [F(1,35) = 7.4, P < 0.01] and IGF-I mRNA in liver [F(1,37) = 7.33, P < 0.01]. There was a significant correlation [r = 0.68; F(1,32) = 27, P < 0.01] between serum concentrations of IGF-I and liver IGF-I mRNA (C). Values shown are means ± SE of 9–11 rats/group. **P < 0.01 and *P < 0.05 vs. control-saline. °P < 0.05 vs. AA-saline.

Fig. 3. Effect of 8-day administration of GHRP-2 (100 μg/kg sc) or saline (250 μl) to control or arthritic rats on IGF-I mRNA in cardiac muscle. A representative Northern blot analysis showing the 7.5, 1.7, and 0.9 kb IGF-I transcripts and the 28S ribosomal RNA in each sample is shown on bottom. Data from 9–10 individual rats were quantified by densitometry and expressed as a percentage of the mean value in control rats treated with saline. Arthritis did not modify IGF-I gene expression in the cardiac muscle. GHRP-2 administration increased IGF-I mRNA in cardiac muscle [F(1,35) = 4.8, P < 0.05].
agonists on the GH-IGF-I system does not last very long. It has been postulated that ghrelin is not physiologically involved in the regulation of GH, since circulating levels of ghrelin are not correlated with those of GH either in physiological or in pathological conditions (52).

Arthritic rats showed an increase in the weight and protein concentration of gastrocnemius muscle after GHRP-2 administration. This suggests a reduction in proteolysis related to a reduction in MuRF1 and MAFbx gene expression. The small increase in serum concentrations in IGF-I along with the decrease in muscular IGFBP-5 and TNF-α after GHRP-2 administration in arthritic rats can contribute to the decrease in MuRF1 and MAFbx gene expression and the increase in skeletal muscle protein.

A wide variety of ghrelin effects has been described as GH independent. Chronic GHRP-2 administration does not modify serum levels of IGF-I, insulin, and glucose, although it increases body weight gain and increases caloric intake (53). Treatment with ghrelin receptor agonists protects the heart from ischemia reperfusion damage (13) through a GH-independent action (38). A protective effect of GHRP-2 administration on proteolysis during critical illness has been previously reported in humans and was not related to the changes observed within the somatotropic axis (57).

The possibility exists that GHRP-2 has a direct effect on the skeletal muscle. Binding of ghrelin agonists in the skeletal muscle have been reported (43). In vitro application of ghrelin agonists or ghrelin modulates the chloride and potassium conductances in rat skeletal muscle, indicating that ghrelin directly affects skeletal muscle function and the presence of ghrelin receptors in this tissue (44). These effects of ghrelin on skeletal muscle are not mediated through the GH/IGF-I axis.

The inhibitory effect of GHRP-2 on TNF-α gene expression in the skeletal muscle is in accordance with other anti-inflammatory effects previously described for ghrelin and synthetic ghrelin receptor agonists. Ghrelin is able to prevent the LPS-induced increase in TNF-α gene expression in the spleen and liver (18). Ghrelin decreases circulating cytokines in pancreatitis (16), in arthritis (24), and in sepsis induced by LPS injection (35). This anti-inflammatory effect is independent of...
pituitary GH secretion, since ghrelin or ghrelin receptor agonists are able to decrease cytokine production in endothelial or immune cells in culture (18, 24, 35, 61). These inhibitory effects of cytokine production in vitro do not seem to be mediated by GH, since this hormone has a stimulatory effect on cytokine activity (17, 56).

In conclusion, this study demonstrates that arthritis induces a marked decrease in skeletal muscle weight along with an activation of the ubiquitin-proteasome proteolytic pathway, whereas administration of GHRP-2, the synthetic ghrelin analog, prevents these effects. Both the effect of arthritis and the GHRP-2 administration on the skeletal muscle can be mediated by changes in muscular IGFBP-5 and TNF-α gene expression.

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