Experimental arthritis inhibits the insulin-like growth factor-I axis and induces muscle wasting through cyclooxygenase-2 activation

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Submitted 15 September 2006; accepted in final form 5 February 2007

Granado M, Martín AI, Villanúa MA, López-Calderón A. Experimental arthritis inhibits the insulin-like growth factor-I axis and induces muscle wasting through cyclooxygenase-2 activation. Am J Physiol Endocrinol Metab 292: E1656–E1665, 2007. First published February 6, 2007; doi:10.1152/ajpendo.00502.2006.—Chronic arthritis induces cachexia associated with an inhibition of the growth hormone (GH)-insulin-like growth factor-I (IGF-I) system and an activation of the E3 ubiquitin-ligating enzymes muscle atrophy F-box (MAFbx) and muscle Ring finger 1 (MuRF1) in the skeletal muscle. The aim of this work was to study the role of cyclooxygenase (COX)-2 in chronic arthritis-induced cachexia. Arthritis was induced in rats by Freund’s adjuvant injection, and the effects of two COX inhibitors (indomethacin, a nonspecific inhibitor, and meloxicam, a selective COX-2 inhibitor on pituitary GH and on liver and serum IGF-I levels) were tested. Arthritis decreased body weight gain and GH and liver IGF-I gene expression. In the arthritic rats, both inhibitors, indomethacin and meloxicam, prevented the inhibitory effect of arthritis on body weight gain. Indomethacin and meloxicam administration to arthritic rats increased pituitary GH and liver IGF-I mRNA as well as serum levels of IGF-I. These data suggest that induction of COX-2 during chronic inflammation is involved in the inhibition of the GH-IGF-I axis and in the body weight loss. In the gastrocnemius muscle, arthritis increased the gene expression of tumor necrosis factor (TNF)-α, the E3 ubiquitin-ligating enzymes MAFbx and MuRF1, as well as of IGF-I and IGF-binding protein-5 (IGFBP-5). Inhibition of COX-2 by meloxicam administration increased gastrocnemius weight and decreased MAFbx, MuRF1, TNF-α, and IGFBP-5 gene expression. In summary, our data indicate that chronic arthritis-induced cachexia and muscle wasting are mediated by the COX-2 pathway resulting in a decreased GH-IGF-I secretion and increased expression of MAFbx and MuRF1 mRNA.

CHRONIC INFLAMMATION IS ASSOCIATED WITH a decrease in body weight and cachexia. One of the numerous factors that can be involved in inflammatory cachexia is the neuroendocrine system. The neuroendocrine response to inflammation is characterized by an increase in the secretion of catabolic hormones such as glucocorticoids and a decreased secretion of anabolic factors such as insulin-like growth factor-I (IGF-I) (24). These modifications, together with the increased release of cytokines, can result in hypermetabolism and a decrease in body weight.

Adjuvant-induced arthritis is an experimental model of rheumatoid arthritis that is induced in rats by an intradermal injection of Freund’s adjuvant. Ten days after adjuvant injection, rats start to lose body weight even before the external signs of the illness are manifested (37). Cachexia and hypermetabolism have also been reported in rheumatoid arthritis patients (52). Rheumatoid cachexia has been postulated as an important contributor in increasing morbidity and premature mortality in rheumatoid arthritis patients (61). Cachexia in experimental arthritis is associated with a decreased secretion of the growth hormone (GH)-IGF-I axis, whereas GH administration to arthritic rats is able to increase body weight gain (28). The increased production of cytokines, mainly tumor necrosis factor-α (TNF-α), seems also to be involved in inflammatory cachexia, since the neutralization of TNF-α in arthritic rats increases body weight gain as well as pituitary GH and liver IGF-I gene expression (21).

Possible factors that can mediate the cachetic effect of TNF-α are prostaglandins. Proinflammatory cytokines induce the production of PGE2, which plays an important role in the inflammatory response. Cyclooxygenases (COXs) are the key enzymes in regulating the biosynthesis of prostaglandins. There are two COX isoforms, encoded by different genes: COX-1 which is constitutively expressed, and COX-2, which is inducible by proinflammatory cytokines and other stimuli. COX-2-induced synthesis of prostaglandins has been associated with chronic inflammation, including arthritis, since the anti-inflammatory effect of selective COX-2 inhibitors is as effective as nonselective inhibitors (17). However, the role of COX-1 in inflammation has also been proposed (13).

Body weight loss in cachexia is secondary to muscle wasting and to enhanced protein breakdown by the ubiquitin-proteasome proteolytic pathway (34). Muscle atrophy F-box (MAFbx) and muscle Ring finger 1 (MuRF1) are muscle-specific E3 ubiquitin-ligating enzymes that play an important role in muscle atrophy and serve as early markers of skeletal muscle atrophy, aiding in the diagnosis of muscle disease. MAFbx overexpression in C2C12 cells induces a decrease in myotube diameter, and mice deficient in MAFbx or MuRF1 are resistant to denervation-induced muscle atrophy (7). In addition, MAFbx and MuRF1 mRNA are increased in atrophying muscle in many inflammatory conditions, including sepsis (63) and chronic arthritis (20).

Proinflammatory cytokines such as interleukin-1 and TNF-α have been reported to increase muscle protein proteolysis by the ubiquitin-proteasome pathway (for review, see Ref. 1). In addition to cytokines, the increased activity of COX can be responsible for muscle wasting and cachexia. Activation of COX-2 pathways has been observed in several cachetic state conditions such as cancer and sepsis (15, 19). COX inhibitors are able to ameliorate cancer cachexia and to improve body composition both in experimental animals and humans (15, 27,
Inhibition of COX activity prevents muscular wasting in cancer and reduces the ubiquitin ligases (25). However, the anticachexic effect of the inhibitors of prostaglandin synthesis has been reported in several types of cancer, whereas they are unable to prevent cancer cachexia in others (23).

The use of nonsteroidal anti-inflammatory drugs (NSAIDS) or unspecific COX inhibitors has possible side effects on gastrointestinal function and hematological tissues, especially in chemotherapy treatments in cancer patients. However, NSAIDS are widely used in inflammatory conditions such as rheumatoid arthritis because of their effective anti-inflammatory activity. Accordingly, the aim of this work was to elucidate the effect of COX inhibition on experimental arthritis-induced cachexia. For that reason, we compared the response in arthritic rats to treatment with an unspecific COX inhibitor, indomethacin, and a COX-2 inhibitor, meloxicam, on body weight, on the GH-IGF-I system, and in the gene expression of negative regulators of IGF-I signaling and of muscular growth, the expression of these two genes were also analyzed.

**MATERIALS AND METHODS**

Arthritic and control male Wistar rats weighing 150–175 g (6 wk old) at the beginning of the experiment were purchased from Charles River (Barcelona, Spain). Arthritis was induced in the rats by an intradermal injection of 1 mg heat-inactivated Mycobacterium butyricum in incomplete Freund’s adjuvant in the right paw. Control animals were injected with mineral oil. Rats were housed 3–4/cage under controlled conditions of light (lights on from 0730 to 1930) and temperature (22 ± 2°C). Food and water were available ad libitum. Assessment of arthritis was performed by measuring the arthritis index of each animal, which was scored by grading each paw from 0 to 4: no erythema or swelling; 1, slight erythema or swelling of one or more digits; 2, swelling of entire paw; 3, erythema and swelling of the ankle; and 4, ankylosis, incapacity to bend the ankle. The severity score was the sum of the clinical scores of each limb, with the maximum value being 16. The procedures followed the guidelines recommended by the European Union for the care and use of laboratory animals, and were approved by the University animal care committee.

**Indomethacin administration.** On day 15 after adjuvant injection, control and arthritic rats were randomly divided into 2 groups; one was injected daily with the unspecific COX inhibitor indomethacin (4 mg/kg sc; Sigma, Madrid, Spain), and the second was injected with vehicle (250 µl of 0.5% NaHCO₃). Indomethacin dose was selected after a study showing that this dose prevents muscle wasting in cancer and weight loss in arthritic rats (25, 58). Body weight, food intake, and the arthritis index scores were examined daily. Food intake per cage was calculated by measuring the difference between the initial and the remaining amount of pellets in the feeder and expressed as gram per rat per 100 g body weight per day. All rats were killed by decapitation 22 days after adjuvant or vehicle injection and, after 8 days of indomethacin 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 assay was performed and at −80°C until PGE₂ analysis was performed. The left hindpaw was amputated at the ankle level, and the volume was measured by water displacement. Immediately after decapitation, the pituitary and the liver were removed, dissected, frozen in liquid nitrogen, and stored at −80°C until RNA extraction.

**Meloxicam administration.** The effect of meloxicam administration, a COX-2 inhibitor, was examined. On day 15, arthritic and control rats were divided into two groups; one was injected with meloxicam (1 mg/kg sc; Sigma), and the second received 250 µl of saline subcutaneously from day 16 to 22. This dosage was selected, since it was reported to be close to an optimal analgesic dose as previously described (54). Rats were injected and weighed, and the arthritis score index and the food intake were examined daily. None of the rats died during the experimental procedures. On day 22 after adjuvant injection, rats were killed, blood was allowed to clot and centrifuged, and the serum was stored until IGF-I and PGE₂ assays were performed. The left hindpaw volume, gastrocnemius muscle, pituitary, kidney, and heart weights were measured. The pituitary, liver, and gastrocnemius muscle were dissected, frozen in liquid nitrogen, and stored at −80°C until RNA extraction.

**Serum IGF-I and PGE₂**. Serum IGF-I concentrations were measured by a double-antibody RIA. Serum IGFBP were removed by...
acid-ethanol extraction. The IGF-I antiserum (UB2-495) was a gift from Dr. L. Underwood and Dr. J. Van Wyk, and it is distributed by the Hormone Distribution Program of National Institute of Diabetes and Digestive and Kidney Diseases through the National Hormone and Pituitary Program. Levels of IGF-I were expressed in terms of IGF-I from Gropep (Adelaide, Australia). The intra-assay coefficient of variation was 8%. Samples from one experiment were run in the same assay.

Serum concentrations of PGE2 were measured by an enzymeimmunoassay system using a commercial kit (Amersham Biosciences, Buckinghamshire, UK) following the manufacturer’s instructions (http://amersham.com).

Real-time PCR. RNA was extracted by the guanidine thiocyanate method using a commercial kit (Ultraspec RNA; Biotecx Laboratories, Houston, TX). The integrity of the RNA was confirmed using agarose gel electrophoresis. For RT-PCR analysis, 2 μg pituitary, liver, or skeletal muscle total RNA were reverse transcribed in a total volume of 30 μl at 37°C for 15 min with 125 units of Moloney murine leukemia virus RT (Maxim Biotech, San Francisco, CA).

Primers for PCR (Table 1), were obtained from Qiagen (Valencia, CA) from previously published sequences of IGF-I, TNF-α and MuRF1 (16), hypoxanthine-guanine phosphoribosyl transferase (Hprt; see Ref. 47), COX-2 (5), or by using the rat GenBank and the EXIQON ProbeLibrary GH, 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 transcripts to minimize amplification).

Each real-time PCR reaction contained 10 ng of synthesized cDNA, 1× Takara SYBR Green Premix Ex Taq (Takara BIO), and 300 nM forward and reverse primers for the candidate gene 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 min, 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 percent of control rats, using the cycle threshold 2^ΔΔCt method (36) with the Hprt as reference gene.

Statistical analysis. All data are presented as means ± SE. Statistics were computed using the statistics program STATGRAPHICS plus for Windows. Differences among experimental groups were analyzed by one-way (organ weights) or two-way ANOVA. Where there were differences among the groups, post hoc comparisons were made by using the unpaired Student’s t-test. Significance was assumed when P < 0.05.

RESULTS

Arthritis induced a significant increase in COX-2 gene expression (P < 0.01) in all the tissues analyzed (pituitary, liver, and skeletal muscle; Fig. 1). As expected, when the serum concentration of PGE2 was measured in arthritic rats treated with two COX inhibitors, both treatments were able to induce a significant (P < 0.01) decrease in the serum levels of PGE2 (Fig. 2).

Three rats from the arthritic group injected with indomethacin died during the experiment, whereas none of the arthritic rats injected with vehicle or meloxicam died during the entire experimental procedure. In the control group injected with indomethacin, one rat died and two rats were excluded from the experiment because they started to lose weight and had ascites. Indomethacin administration to arthritic rats dramatically decreased arthritis index scores (Fig. 3), being significantly lower than in the arthritic rats injected with vehicle on the 3rd day of indomethacin treatment (and 17 days after adjuvant injection). Arthritis increased the paw volume (P < 0.01), and indomethacin treatment reduced paw volume in arthritic rats (P < 0.01, Fig. 3), whereas indomethacin administration had no effect on paw volume in control rats. The anti-inflammatory effect of treatment with meloxicam was similar to that of indomethacin (Fig. 4). Meloxicam administration decreased the external signs of the arthritis, measured as arthritis scores, from the 1st day of treatment. This difference was significant, starting from the 3rd day of treatment (Fig. 4). Meloxicam administration for 8 days also decreased the paw volume in the arthritic rats (P < 0.01) but not in controls.

The effect of arthritis and indomethacin administration on body weight and food intake is shown in Fig. 5. Arthritic rats

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**Fig. 2.** Serum concentration of PGE2 in arthritic rats treated with the COX inhibitors indomethacin and meloxicam (gray bars) or with vehicle (white bars). Both inhibitors decreased the serum concentrations of PGE2 in arthritic rats. Values shown are means ± SE for 8–10 rats/group. **P < 0.01** vs. respective group injected with vehicle.

**Fig. 3.** Evolution of arthritis scores (left) and paw volume (right) in arthritic rats (AA) or control rats injected with indomethacin (4 mg/kg sc) or vehicle (250 μl) for 8 days (treatments started 15 days after adjuvant injection). Values shown are means ± SE (n = 8–10 rats). There was an interaction between the effect of arthritis and indomethacin on paw volume [F(1,31) = 11, P < 0.01], since indomethacin decreased paw volume in arthritic rats but not in control rats. **P < 0.01** vs. control rats injected with vehicle. **P < 0.01** vs. control rats injected with indomethacin. **P < 0.05** and **P < 0.01** vs. arthritic rats injected with vehicle.

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had lower body weight than control rats when starting the indomethacin treatment 15 days after adjuvant injection. In the arthritic rats injected with vehicle, the body weight gain during the 8 days of treatment was lower than that of control rats injected with vehicle ($P < 0.01$). In both experiments, arthritis decreased ($P < 0.01$) the cumulative food intake during the 8 days of treatment (Figs. 5 and 6), but this effect was not significant during the last 2 days of treatment before the rats were killed (Figs. 5 and 6). Indomethacin induced a significant increase in body weight in arthritic rats starting 24 h after administration. Moreover, indomethacin prevented the inhibitory effect of arthritis on body weight gain, since the arthritic rats injected with indomethacin had a cumulative body weight similar to that of control rats injected with vehicle, although the absolute body weight remained lower than the control rats injected with vehicle (Fig. 5). In contrast, indomethacin administration to nonarthritic rats induced a decrease in food intake ($P < 0.01$, Fig. 5) and in body weight gain, where the cumulative body weight gain during the 8 days of treatment was lower than that of the arthritic rats injected with indomethacin (Fig. 5).

The effect of meloxicam administration on body weight and food intake is shown in Fig. 6. Meloxicam administration to arthritic rats prevented the inhibitory effect of arthritis on body weight, since the evolution of body weight during the 8 days of treatment in the arthritic rats injected with meloxicam was similar to that of the control rats. Meloxicam administration had no appreciable effect on food intake in control rats but induced a marked increase in food intake in arthritic rats ($P < 0.01$), even at higher levels than the food intake observed in control rats (Fig. 6).

In the group treated with vehicle, arthritis induced a significant decrease in pituitary GH ($P < 0.05$) and liver IGF-I ($P < 0.01$) gene expression, as well as in serum concentrations of...
IGF-I (P < 0.01, Fig. 7). As observed with food intake and body weight, indomethacin administration decreased serum concentrations of IGF-I and IGF-I gene expression in the liver (P < 0.01) in control rats. There was a significant decrease in serum IGF-I levels in the control rats that received indomethacin. In contrast, in arthritic rats, indomethacin administration increased serum concentrations of IGF-I (P < 0.05) and liver IGF-I mRNA, although this increase was not statistically significant.

Fig. 6. Effect of 8-day meloxicam administration (1 mg/kg sc) on cumulative body weight gain (A), absolute body weight evolution (B), and daily food intake during the 8 days (C) or during the last 2 days (D) of treatment in control or arthritic (AA) rats. There was an interaction between the effect of arthritis and meloxicam on body weight gain [F(1,36) = 25, P < 0.01] and on food intake during the 8 days [F(1,24) = 16, P < 0.01] or during the last 2 days [F(1,20) = 44, P < 0.01] of treatment, since meloxicam increased the body weight gain and food intake in arthritic rats but not in control rats. Values represent means ± SE for n = 4–12. *P < 0.05 and **P < 0.01 vs. control rats injected with vehicle. °P < 0.05 and °°P < 0.01 vs. arthritic rats injected with vehicle.

Fig. 7. Effect of indomethacin administration over 8 days on pituitary growth hormone (GH) mRNA (A), liver insulin-like growth factor (IGF)-I mRNA (B), and on serum concentrations of IGF-I (C) in control or arthritic (AA) rats. mRNAs were quantified using real-time RT-PCR as described in MATERIALS AND METHODS and are presented as a percentage of the mean value in the control group injected with vehicle. There was an interaction between the effect of indomethacin and arthritis on liver IGF-I mRNA [F(1,24) = 11, P < 0.01] and on serum concentrations of IGF-I [F(1,32) = 17, P < 0.01], since arthritis decreased circulating IGF-I and its gene expression in the liver in vehicle-injected rats but not in the rats treated with indomethacin. Values represent means ± SE for n = 7–10. *P < 0.05 and **P < 0.01 vs. control rats injected with vehicle. °P < 0.05 vs. arthritic rats injected with vehicle.
significant. Indomethacin treatment did not modify pituitary GH mRNA in control rats but blunted the inhibitory effect of arthritis on pituitary GH mRNA.

In contrast to the data obtained with the indomethacin treatment, meloxicam administration to control rats did not modify liver IGF-I mRNA or serum concentrations of IGF-I (Fig. 8). In arthritic rats, meloxicam administration increased the serum concentration of IGF-I as well as its gene expression in the liver, reaching levels similar to those observed in the control rats (Fig. 8). Similarly, meloxicam administration also blunted the inhibitory effect of arthritis on GH mRNA (Fig. 8).

Because of the observed side effects of indomethacin, and taking into account that in the arthritic rats the effect of indomethacin and meloxicam on body weight and on the GH-IGF-I axis was similar, we studied the skeletal muscle response only in the rats treated with meloxicam. The effect of arthritis and meloxicam administration to arthritic rats on gastrocnemius, heart, kidney, and pituitary weights is shown in Fig. 9. Arthritis decreased the weight of all organs studied, but there were differences among them. Arthritis induced a dramatic decrease in skeletal muscle, where the gastrocnemius was 28% \( (P < 0.01) \) of gastrocnemius weight of the control rats injected with saline. The weight of organs such as the heart, kidney, and pituitary were also decreased by arthritis, but their decreases were lower (73–84% of control values) than that of the skeletal muscle decrease. In the control rats, meloxicam administration had no effect in all the organs analyzed (data not shown). These data indicate that the inhibitory effect of arthritis on body weight is mainly the result of skeletal muscle atrophy. Similarly, the stimulatory effect of meloxicam on body weight in arthritic rats is concomitant with an increase in the weight of the organs studied, where this effect is more marked in skeletal muscle weights than in kidney, heart, or pituitary weights (Fig. 9).

The effect of arthritis on IGF-I gene expression in the gastrocnemius is different from the liver, since IGF-I mRNA was higher \( (P < 0.05) \) in arthritic rats than in control rats injected with saline (Fig. 10). Meloxicam treatment, although it increased IGF-I mRNA in the gastrocnemius, had no statistical significance in control or in arthritic rats. Arthritis also induced an increase \( (P < 0.01) \) in IGFBP-5 mRNA in the gastrocnemius (Fig. 10). Meloxicam administration did not modify the IGFBP-5 mRNA in the gastrocnemius of control rats, whereas it decreased the IGFBP-5 mRNA in the arthritic rats, reaching levels similar to those of control rats.

The effect of meloxicam administration on MAFbx and MuRF1 gene expression in the gastrocnemius of control or arthritic rats is shown in Fig. 11. In the control rats, meloxicam administration did not modify either MAFbx or MuRF1 mRNAs. Arthritis induced a significant increase in MAFbx \( (P < 0.01) \) and MuRF1 \( (P < 0.05) \) mRNAs in the gastrocnemius.

![Figure 8](http://ajpendo.physiology.org/)

**Fig. 8.** Effect of meloxicam treatment over 8 days on pituitary GH mRNA (A), liver IGF-I mRNA (B), and on serum concentrations of IGF-I (C) in control or arthritic (AA) rats. mRNAs were quantified using real-time RT-PCR as described in MATERIALS AND METHODS and are presented as percentage of the mean value in the control group injected with vehicle. There was an interaction between the effect of meloxicam and arthritis on serum concentrations of IGF-I \( F(1,35) = 5, P < 0.05 \), since arthritis decreased the serum concentration of IGF-I in the rats treated with saline but not in the group treated with meloxicam. Values represent means \( \pm SE \) for \( n = 7–10 \). *\( P < 0.05 \) and **\( P < 0.01 \) vs. control rats injected with vehicle. **\( P < 0.05 \) vs. arthritic rats injected with vehicle.

![Figure 9](http://ajpendo.physiology.org/)

**Fig. 9.** Weight of gastrocnemius, heart, kidney, and pituitary of arthritic rats injected with vehicle (AA-vehicle) or meloxicam (AA-meloxicam, 1 mg/kg sc). Data are expressed as % weight of the control rats injected with vehicle for 10 rats/group. \( P < 0.01 \) vs. control rats injected with vehicle (***) and vs. arthritic rats injected with vehicle (**).
mius, and meloxicam treatment totally prevented the effect of arthritis on these mRNAs (Fig. 11).

Arthritis also induced an increase on TNF-α gene expression in skeletal muscle (Fig. 12). Meloxicam administration did not modify the TNF-α mRNA in control rats, whereas in arthritic rats meloxicam induced a significant decrease in TNF-α mRNA, reaching control values.

**DISCUSSION**

Our data show that arthritis increases COX-2 gene expression, whereas inhibition of COX-2 activity prevents arthritis-induced muscle wasting and the increase in the E3 ubiquitin-ligating enzymes MAFbx and MuRF1. These effects are associated with an amelioration of the GH-IGF-I axis and a decrease in the gene expression of IGFBP-5 and TNF-α in the skeletal muscle, taking into account that the anti-inflammatory effect of both treatments (indomethacin, a nonselective inhibitor, and meloxicam, a preferential selective COX-2 inhibitor) was similar. The inhibitory effect of arthritis on the GH-IGF-I system and on body and skeletal muscle weights seems to be mediated through activation of the COX-2 pathway.

The antianorexigenic effect of COX inhibitors is well known, and it has been postulated that eicosanoids are more important in anorexia than host cytokines (51). In the present data, the two COX inhibitors increased food intake and body weight gain in the arthritic rats. The antianorexigenic effect of indomethacin in the arthritic rats contrasts with its effect in control rats in which indomethacin decreased food intake, body

![Fig. 10. Effect of meloxicam administration on IGF-I (top) or IGFBP-5 mRNA (bottom) in the gastrocnemius of control or arthritic (AA) rats. mRNAs were quantified using real-time RT-PCR as described in MATERIALS AND METHODS and are presented as %mean value in control group injected with vehicle. There was an interaction between the effect of arthritis and meloxicam administration on IGF-I mRNA [F(1,32) = 7.2, P < 0.05], since arthritis increased IGF-I mRNA in the gastrocnemius of the rats injected with vehicle but not in those injected with meloxicam. Results are expressed as means ± SE for n = 8–10/group. *P < 0.05 and **P < 0.01 vs. control rats injected with vehicle. **P < 0.01 vs. arthritic rats injected with vehicle.](http://ajpendo.physiology.org/)
weight gain, IGF-I gene expression in the liver, and circulating IGF-I. Toxicity to indomethacin but not to meloxicam was observed, as indicated by mortality and a decrease in body weight gain in the control rats injected with indomethacin. A toxic effect of 5 mg/kg indomethacin has previously been reported, but without mortality (17). In arthritic rats undergoing treatment with a lower indomethacin dosage (3 mg/kg), one group did not report having adverse effects of indomethacin (49), whereas another one reported mortality (58). Indomethacin side effects have been attributed to gastrointestinal damage because of COX-1 inhibition. A detrimental effect of a COX-1 inhibitor on body weight has been reported, since COX-1 inhibition or genetic inactivation of COX-1 augmented and prolonged body weight loss during inflammation (29). In control rats injected with indomethacin, the gastrointestinal damage can be the cause of the decrease in food intake. The decrease in body weight gain and in IGF-I gene expression in the control rats injected with indomethacin can be related to the decrease in food intake. According to this, pituitary GH gene expression is not affected by indomethacin in the control rats, since GH is not as related to food intake as IGF-I is.

Although the rats in this study were not pair-fed, we have previously found that the inhibitory effect of arthritis on body weight and IGF-I is not the result of anorexia, since pair-fed rats gained similar body weight to control rats fed “ad libitum” (38). A possible explanation is that the anorexic effect of arthritis is small, the food intake is 90% that of the control rats, whereas the body weight gain is 11% of the control rats during the 8 days of the experiment. In addition, there is no difference in food intake from day 21 to 23 (the last 2 days before the animals died) between arthritic and control rats injected with vehicle. Furthermore, recombinant human GH administration to arthritic rats increased body weight gain without increasing food intake (28). On the contrary, anti-TNF-α administration to arthritic rats increases food intake to control levels, but the body weight gain was still lower than the control rats (21). In cancer, it has been reported that initially body wasting occurs independently of anorexia (6, 25, 62). In other cachectic conditions such as lipopolysaccharide (LPS) administration, the mechanism by which LPS reduces food intake is also dissociated from its effect on weight loss (42). All these data indicate that, in addition to the decrease in food intake, the decrease in body weight in arthritic rats is related to other mechanisms.

The effect of both COX inhibitors on body weight in the arthritic rats can be secondary to its effect on GH and IGF-I, since the immunosuppressors cyclosporine A blocks the inhibitory effect of arthritis on circulating GH and IGF-I and also prevents the inhibitory effect of arthritis on body weight (59). Taking into account that exogenous GH is able to counteract the decline in skeletal muscle function in chronic cardiac failure (14), part of the effect of meloxicam in preventing cachexia can be secondary to the increased gene expression on GH and liver and serum IGF-I. The inhibitory effect of arthritis on GH does not seem to be mediated by the increased release of PGE_2, since this prostanoid has been reported to be a GH secretagogue (12, 43). A stimulatory effect of lipoxigenase products on pituitary GH synthesis and secretion has also been reported (12, 53). Therefore, the inhibitory effect of arthritis on pituitary GH can be secondary to another inhibitory substance that is released after COX-2 activation. In the liver, COX-2 upregulation potentiates liver injury induced by allyl alcohol, where PGD_2, but not PGE_2, has a similar effect in isolated hepatocytes (19). A direct inhibitory effect of PGA and -J on IGF-I gene expression has been reported (9, 10), whereas PGE_2 has a stimulatory effect on IGF-I gene expression in osteoblasts (8, 54). Therefore, it is also possible that another prostaglandin different from PGE_2 inhibits the pituitary GH and liver IGF-I during inflammation.

IGF-I has a prominent role in muscle hypertrophy, but when chronically deactivated it also has an important role in muscle atrophy (31). Accordingly, the increased IGF-I mRNA in skeletal muscle of arthritic rats contrasts with the notable muscle wasting induced by arthritis. One possible explanation for this fact is that muscular IGFBP-5 might inhibit IGF-I activity in skeletal muscle by preventing its interaction with the IGF-I receptor. In addition to its IGF-I-dependent action, IGFBP-5 is also able to decrease cell proliferation by an IGF-I-independent effect in myoblast cultures (44). Similarly, transgenic mice overexpressing IGFBP-5 show growth retardation and decreased skeletal muscle mass relative to body weight (57). In the present data, COX-2 inhibition in arthritic rats decreased IGFBP-5 mRNA in the skeletal muscle. It is possible that meloxicam directly decreases IGFBP-5 gene expression by decreasing prostanoit biosynthesis in skeletal muscle. A stimulatory effect of PGE_2 on IGFBP-5 in osteoblast cells has also been described (46). Moreover, PGE_2-responsive elements have been reported in the IGFBP-5 promoter (4, 40). Changes in pituitary GH secretion can also modify muscular IGFBP-5 mRNA, since GH inhibits the expression of IGFBP-5 in mammary epithelial cells (56).

Local COX-2 pathways can play an important role in muscle wasting. Arthritis increases COX-2 and specific E3 ubiquitin-ligating enzyme, MAFbx and MuRF1, gene expression in the muscle. In septic patients, an increase in COX-2 and activation of the ubiquitin proteolytic pathway in muscle have been reported (50). In addition, COX-2 inhibition by celecoxib decreases muscle weakness in elderly patients with acute inflammation (35). The preservation of muscle mass in the arthritic rats injected with meloxicam was associated with a decrease in the muscular expression of both atrogens. These results suggest that the COX-2 pathway is involved in the upregulation of MAFbx and MuRF1 during chronic arthritis. Taking into account that these genes are considered as markers of skeletal muscle atrophy, our data suggest that the COX-2 pathway is involved in muscular wasting.

It is possible that the improvement in mobility, rather than the decrease in prostaglandins per se, may lead to the observed changes in gene expression in the gastrocnemius. We cannot exclude this possibility, because we did not measure mobility. However, this does not seem to be the cause, since with systemic anti-TNF-α administration, inflammation was also decreased in a similar way (21), but no modifications in IGFBP-5, MAFbx or MuRF1 mRNA, or gastrocnemius weight were observed (22). Taking into account that TNF-α is also an important trigger of muscular atrophy and it upregulates MAFbx and MuRF1 gene expression (31, 35), the lack of effect of systemic anti-TNF-α in preventing muscular wasting was unexpected (22). A possible explanation can be that the “in vivo” anti-TNF-α therapy is not able to suppress COX activity in the muscle, as is the case of the synovial tissue from rheumatoid arthritis patients (33). These data emphasize the
importance of the COX pathway in the inflammatory cachexia. Another explanation could be that muscle proteolysis by the ubiquitin-proteasome pathway is activated by local TNF-α rather than by circulating TNF-α, since systemic anti-TNF-α therapy is not able to prevent the increased gene expression of TNF-α in the gastrocnemius muscle (22). In the present data, normalization of MuRF1 and MAFbx is associated with a decrease in muscular TNF-α, which means that there is a relationship between prostaglandin II synthesis and TNF-α gene expression. Supporting these data, normalization of TNF-α gene expression by NSAID administration has previously been reported in cancer (32), in endotoxemic rats (3), in diabetic retinopathy (30), and has been explained by the inhibitory effect of the NSAIDs on NF-kB signaling.

Nuclear factor (NF) is responsible for chronic inflammation-induced cachexia. As a central component of the muscular atrophy process involved in the activation of proteolytic pathways. Taking into account that meloxicam inhibits the activation of NF-κB (2, 30, 64), this can be one of the mechanisms by which meloxicam prevented the arthritis-induced increase of MAFbx and MuRF1 expression and muscle wasting.

In conclusion, our data suggest that overactivation of COX-2 is responsible for chronic inflammation-induced cachexia, through an inhibition of the GH-IGF-I axis and an activation of the ubiquitin-proteasome pathway and muscle wasting. At the muscular level, COX-2 seems to be involved in the increase in local IGFBP-5 and TNF-α gene expression that contributes to the activation of the MAFbxs and MuRF1 ubiquitin ligases. These findings have implications not only on the knowledge of the mechanism leading to cachexia in chronic inflammation but also on therapeutic strategies in managing chronic arthritis.

ACKNOWLEDGMENTS

We are indebted to Antonio Carmona for technical assistance and to Christina Bickart for the English correction of the manuscript.

GRANTS

This work was supported by Programa Nacional de Promoción General del Conocimiento, Plan Nacional de Investigación Científica, from Ministerio de Educación Ciencia (BFI-2003-02149), by Universidad Complutense/Santander-CentralHispano Grant PR27/05-14055, and a Fellowship from Ministerio de Educación y Ciencia to M. Granado (FPU, AP2003-2564).

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