Fenofibrate, a PPARα agonist, decreases atrogens and myostatin expression and improves arthritis-induced skeletal muscle atrophy

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Cachexia is a complex syndrome associated with an activation of inflammatory response, body weight loss, skeletal muscle wasting, and sometimes loss of adipose tissue mass (17). Adjuvant arthritis is an animal model of rheumatoid arthritis that can be induced in rats by an intraderal injection of Freund’s adjuvant. Ten days after injection, arthritic rats start to develop the external signs of the illness, a decrease in body weight gain together with adipose tissue and skeletal muscle wasting (10, 34). Similarly, cachexia and hypermetabolism have also been reported in rheumatoid arthritis patients, and cachexia has been postulated as an important contributor in increasing morbidity and mortality (52). Although experimental arthritis induces anorexia, muscle wasting is not due to the decrease in food intake (10). One of the mechanisms by which inflammation, including arthritis, induces muscle wasting is by activation of the ubiquitin-proteasome pathway (24). The muscle-specific E3 ubiquitin-ligating enzymes atrogin 1 and muscle RING finger 1 (MuRF1) play an important role in muscle atrophy and serve as early markers of skeletal muscle atrophy, aiding in the diagnosis of muscle disease (30). Atrogin-1 expression is under control of Forkhead box O (FoxO) transcription factors, and cachetic stimuli such as endotoxin or glucocorticoid administration activate FoxO3 by reducing the activity of Akt (14, 57).

Peroxisome proliferator-activated receptors (PPARs) are transcription factors that regulate genes involved in energy homeostasis through control of lipid and glucose metabolism (for review, see Ref. 39). Three different isoforms of PPARs have been identified to date, PPARα, PPARβ/δ, and PPARγ. In addition to their actions on lipid metabolism, PPARs and PPARγ inhibit the inflammatory response in vascular and inflammatory cells (15). It has been shown that oral administration of the PPARα agonist gemfibrozil can prevent the development of experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis (22). Furthermore, when PPARα agonists were used clinically as treatment for dyslipidemia in rheumatoid arthritis patients, anti-inflammatory effects of these drugs were also observed (43). In addition, fenofibrate, a ligand for PPARα, inhibits cytokine production from rheumatoid synovial fibroblasts as well as osteoclast differentiation from osteoclast progenitor cells (44). Those authors also found that fenofibrate inhibits the development of adjuvant-induced arthritis in female Lewis rats (44).

Activation of PPARs is exerted by a wide range of synthetic and endogenous ligands. ω-3 Polyunsaturated fatty acids have beneficial effects on cardiovascular health and on inflammatory diseases. Eicosapentaenoic acid (EPA) is an ω-3 polyunsaturated fatty acid that is a common component of fish oil and a PPAR activator. Several clinical studies have shown benefits from fish oil and EPA in patients with rheumatoid arthritis (26, 50). In arthritic rats, we have reported that EPA administration decreases atrogens and increases muscle mass (51). In addition, EPA administration decreases atrogens and increases muscle mass (51).

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References

1. Castillero E, Nieto-Bona MP, Fernández-Galaz C, Martín AI, López-Menduiña M, Granado M, Villanúa MA, López-Calderón A. Fenofibrate, a PPARα agonist, decreases atrogens and myostatin expression and improves arthritis-induced skeletal muscle atrophy. Am J Physiol Endocrinol Metab 300: E790–E799, 2011. First published February 8, 2011; doi:10.1152/ajpendo.00590.2010.—Arthritis is a chronic inflammatory illness that induces cachexia, which has a direct impact on morbidity and mortality. PPARα, a selective PPAR agonist prescribed to treat human dyslipidemia, has been reported to decrease inflammation in rheumatoid arthritis patients. The aim of this study was to elucidate whether fenofibrate is able to ameliorate skeletal muscle wasting in adjuvant-induced arthritis, an experimental model of rheumatoid arthritis. On day 4 after adjuvant injection, control and arthritic rats were treated with 300 mg/kg fenofibrate until day 15, when all rats were euthanized. Fenofibrate decreased external signs of arthritis and liver TNFα and blocked arthritis-induced decrease in PPARα expression in the gastrocnemius muscle. Arthritis decreased gastrocnemius weight, which results from a decrease in cross-section area and myofiber size, whereas fenofibrate administration to arthritic rats attenuated the decrease in both gastrocnemius weight and fast myofiber size. Fenofibrate treatment prevented arthritis-induced increase in atrogin-1 and MuRF1 expression in the gastrocnemius. Neither arthritis nor fenofibrate administration modify Akt-FoxO3 signaling. Myostatin expression was not modified by arthritis, but fenofibrate decreased myostatin expression in the gastrocnemius of arthritic rats. Arthritis increased muscle expression of MyoD, PCNA, and myogenin in the rats treated with vehicle but not in those treated with fenofibrate. The results indicate that, in experimental arthritis, fenofibrate decreases skeletal muscle atrophy through inhibition of the ubiquitin-proteasome system and myostatin.

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fenofibrate, on arthritis-induced muscle wasting was studied. We report herein the effectiveness of fenofibrate administration in increasing gastrocnemius mass, preventing arthritis-induced upregulation of the two ubiquitin ligases atrogin-1 and muscle RING finger 1 (MuRF1), as well as decreasing myostatin expression in the gastrocnemius muscle. This represents a novel effect of the PPARα activator fenofibrate.

**MATERIALS AND METHODS**

**Animals and treatments.** Control and arthritic male Wistar rats (150 g) were purchased from Charles River Laboratories (Barcelona, Spain). Arthritis was induced in rats by an intradermal injection in the right paw of 4 mg of Freund’s adjuvant (heat-inactivated Mycobacterium butyricum) under isoflurane anesthesia. Upon arrival (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 0730 to 1930) with food and water available ad libitum. All procedures were carried out in accordance with the guidelines recommended by the European Union for the care and use of laboratory animals and were approved by the Complutense University Animal Care Committee.

**Experimental design.** On day 4 after adjuvant injection, control and arthritic rats were divided into two groups (n = 16 rats/group). The first group received fenofibrate (300 mg/kg body wt suspended in 500 μl of 1% carboxymethylcellulose; Sigma-Aldrich, Madrid, Spain) daily by oral gavage. The second group was gavaged with vehicle (500 μl, 1% carboxymethylcellulose). A pair-fed group of 16 rats treated with vehicle was also included; it received the same amount of food (g/100g body wt) eaten by arthritic rats treated with vehicle the day before. Body weight, food intake, and arthritis severity were assessed daily. Evaluation of arthritis severity was performed by measuring the arthritis index of each animal, which was clinically scored by grading each paw from 0 to 4, as described previously (49). Grading was determined as follows: 0, no erythema or swelling; 1, slight erythema or swelling of one or more digits; 2, swelling of the entire paw; 3, erythema and swelling of the ankle; 4, ankylosis, incapacity to bend the ankle. The severity score was the sum of the clinical scores of the four limbs, the maximum value being 16. After 11 days of treatment, on day 15 after adjuvant injection, all rats were euthanized by decapitation between 1200 and 1300. Spleens and left gastrocnemius muscles were removed and weighed. The left hindpaws were amputated at the ankle level, and their volume was measured by water displacement. Left gastrocnemius and liver of nine rats of each group were dissected, frozen in liquid nitrogen, and stored at −80°C.

**Skeletal muscle morphology.** Left gastrocnemius (n = 7/group) was weighed, and the medial part was dissected, placed over a transparency film, glued at one end to a cork with gum tragacanth (Fibraguari; Fardi, Madrid, Spain), frozen in isopentane, cooled by liquid nitrogen, and stored at −80°C. Ten-micrometer cryostat sections taken from the midbelly region were fixed with 100% acetone and stained with hematoxylin-eosin. Parallel sections were kept at −80°C until further processing for immunohistochemical analysis. Four to six muscle hematoxylin-eosin-stained sections were used to determine the whole cross-sectional area. Sections were scanned (Epson Scanner 4990) with a transparent rule, and the area was measured with Image J software.

We used muscle fiber cross-sectional size as an index of type-specific fiber atrophy. Slow muscle fibers were detected with a monoclonal antibody against slow myosin heavy-chain form (1:80; NCL-MHCs-Novocastra, Newcastle Upon Tyne, UK) and secondary Alexa fluor 488 goat antimouse IgG (1:100, A11001-Invitrogen; Invitrogen, Madrid, Spain). The extracellular matrix was detected by Wheat Germ Agglutinin labeled with Texas Red (W849, 1 g/ml; Invitrogen). Sections were mounted with Prolong-Gold antifade reagent combined with 4,6-di-amidino-2-phenylindole (P36931; Invitrogen). Digital images were acquired with a Leica DMi300 microscope. Fiber boundaries were detected from the wheat germ agglutinin fluorescent images using Difference of Gaussians algorithm by GIMP software. At least two images of the medial area (mixed area with slow and fast fibers) and two
images of the lateral area (only fast fibers) from each section were used to measure the slow and fast fiber minimal Feret’s diameter (7) with Image J software. Feret’s diameter was used as a measurement of size because it is more resistant to variation in orientation than cross-sectional area.

RNA extraction and real-time PCR. RNA was extracted using UltraspecTM (Biotecx Laboratories, Houston, TX). 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 using Quantiscript Reverse Transcription kit (Qiagen Combh Hilden, Valencia, CA). Real-time PCR for quantification of mRNA was performed on a SmartCycler (Cepheid, Sunnyvale, CA), using a SYBR Green protocol on the fluorescence temperature cycler. Each real-time PCR reaction consisted of 10 ng of total RNA equivalents, 1× Takara SYBR Green Premix Ex Taq (Takara Bio, Otsu, Shiga, Japan), and 300 nM forward and reverse primers in a reaction volume of 25.5 µl. Primers for real-time PCR were obtained from Roche (Madrid, Spain) by using the EXIQON Universal Probe Library, as described previously (10). The thermal cycling profile consisted of a preincubation step at 95°C for 10 s followed by 40 cycles of 95°C denaturation steps for 15 s, 60°C annealing steps for 30 s, and 72°C extension steps for 30 s. Results were expressed as fold changes in the expression of each gene in arthritic and pair-fed rats compared with control animals treated with vehicle using the cycle threshold 2ΔΔCT method with 18S as reference gene.

Immunoblot. Gastrocnemius was homogenized in RIPA buffer (10 µl/mg) with protease inhibitor cocktail (Sigma-Aldrich) and with phosphatase inhibitors for p-Akt/Akt and p-FoxO3/FoxO3. Protein concentration was determined using the Bradford protein assay (6). The protein extracts were boiled for 5 min in a 1:1 volume of Laemmli loading buffer. Proteins (50 µg) were resolved by electrophoresis on 14% polyacrylamide gels under reducing conditions and transferred onto nitrocellulose membranes that were blocked by incubation in 5% nonfat dry milk and 0.1% Tween (Sigma-Aldrich) in Tris-buffered saline. Ponceau-S staining was performed to ensure equal transfer prior to blocking. Membranes were probed overnight at 4°C sequentially with antibodies against myogenin, PCNA, myostatin, MyoD, FoxO3 (Santa Cruz Biotechnology, Santa Cruz, CA), p-Akt, Akt, p-FoxO3 (Cell Signaling Technology, Boston, MA), and α-tubulin.

Fig. 2. Effect of arthritis and feno (300 mg/kg) on peroxisome proliferator-activated receptor-α (PPARα) mRNA in liver (A) and in gastrocnemius (B). mRNA were quantified using real-time PCR and are presented in relation to the mean value in control group. AA rats have lower liver PPARα than control rats. Gastrocnemius PPARα mRNA was lower in arthritic than in PF rats. Feno increased PPARα in control and in arthritic rats. Data represent means ± SE (n = 7–9 rats). **P < 0.01 vs. control rats; ###P < 0.01 vs. AA rats treated with vehicle; +P < 0.05 vs. PF rats.

Fig. 3. Effect of feno (300 mg/kg) on body weight (A), food intake (B), gastrocnemius (C), and epididymal white adipose tissue (WAT) weight (D). Arthritis decreased body weight and food intake. Feno increased body weight gain, food intake, and gastrocnemius weight in AA rats. Data represent means ± SE (n = 6 cages or 9–16 rats). *P < 0.05; **P < 0.01 vs. control rats; #P < 0.05; ###P < 0.01 vs. AA rats treated with vehicle; +P < 0.01 vs. PF rats; **P < 0.01 vs. control rats treated with feno.
Sigma-Aldrich) with stripping of membranes, using stripping buffer (1 M Tris·HCl, 10% SDS, β-mercaptoethanol) for 30 min at 65°C before each new antibody. Membranes were 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 anti-goat IgG (Thermo Scientific, Rockford, IL)], and peroxidase activity was detected using enhanced chemiluminescent reagent (Amersham Biosciences). Band intensities were quantified by densitometry using Gene Tools Analysis software. The density of the protein band in each lane was expressed as the percentage of the mean density of control rats after normalization for loading using β-tubulin.

**Serum measurements.** Triglycerides, HDL cholesterol, and LDL cholesterol were measured using kits from Spinreact (Sant Esteve de Bas, Spain), following the manufacturer’s protocols.

**Statistical analysis.** Results were compared using the statistics program STATGRAPHICS plus for Windows. Data are presented as means ± SE. Statistical evaluation of the data was performed by one-way analysis of variance; post hoc comparisons were made using the least significant difference multiple range test. Liver TNFα and gastrocnemius PPARα mRNA data were subjected to log transformation, since variances showed a log-normal distribution. Arthritis score index was analyzed by impaired Student’s t-test. Statistical significance was set at P<0.05.

**RESULTS**

**Anti-inflammatory effects of fenofibrate.** As shown in Fig. 1A, on day 10 after adjuvant injection, the external signs of arthritis started to increase, having the maximum values on days 14 and Table 1. Effect of arthritis and fenofibrate (300 mg/kg) on serum triglycerides and lipoprotein levels

<table>
<thead>
<tr>
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<th>C</th>
<th>C + Feno</th>
<th>AA</th>
<th>AA + Feno</th>
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<tr>
<td>Triglycerides, mg/dl</td>
<td>137.7 ± 19.2</td>
<td>72.2 ± 6.3**</td>
<td>73.0 ± 7.6**, +</td>
<td>63.3 ± 7.7 +</td>
<td>116.0 ± 15.9</td>
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<td>HDL cholesterol, mg/dl</td>
<td>33.8 ± 5.5</td>
<td>45.8 ± 5.1</td>
<td>33.4 ± 3.5</td>
<td>46.9 ± 4.1#</td>
<td>37.8 ± 6.5</td>
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<tr>
<td>LDL cholesterol, mg/dl</td>
<td>40.5 ± 2.9</td>
<td>32.6 ± 1.8**</td>
<td>47.5 ± 2.3**</td>
<td>34.6 ± 2.5##</td>
<td>37.6 ± 3.4</td>
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<tr>
<td>HDL/LDL ratio</td>
<td>0.81 ± 0.1</td>
<td>1.41 ± 0.16**</td>
<td>0.75 ± 0.1</td>
<td>1.41 ± 0.12##</td>
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Data represent means ± SE (n = 14–16 rats). C, control; feno, fenofibrate; AA, arthritic; PF, pair fed. Arthritis decreased triglycerides. Feno treatment decreased triglycerides and LDL cholesterol in C rats. In AA rats, feno treatment increased HDL cholesterol, whereas it decreased LDL cholesterol. The HDL/LDL ratio was increased by feno administration in both C and AA rats. **P<0.01 vs. C rats; #P<0.05; ##P<0.01 vs. AA rats treated with vehicle; +P<0.05; + +P<0.01 vs. PF rats.

Statistical analysis. Feno increased fast fiber size in AA rats. Data represent means ± SE (n = 4–6). **P<0.01 vs. control rats; +P<0.05 vs. PF rats; ##P<0.01 vs. AA rats treated with vehicle; **°°P<0.01 vs. control rats treated with feno.

Fig. 4. Effect of arthritis and feno (300 mg/kg) on gastrocnemius cross-sectional area and myofiber size. A: representative cross-sections of the midbelly region of the gastrocnemius. Slow fibers are shown in red. B: average cross-sectional areas of gastrocnemius from control, AA, or PF rats. C: representative transverse sections from mix region (left) or from fast fiber region (right) of gastrocnemius muscle immunostained with type I (slow) myosin heavy-chain antibody (green). All fibers are outlined by wheat germ agglutinin (red). Nuclei were tagged using 4,6-diamidino-2-phenylindole (blue). D and E: slow and fast fiber diameter, respectively. Arthritis decreased gastrocnemius cross-sectional area as well as fast and slow fiber size. Feno increased fast fiber size in AA rats. Data represent means ± SE (n = 4–6). **P<0.01 vs. control rats; +P<0.05 vs. PF rats; ##P<0.01 vs. AA rats treated with vehicle; **°°P<0.01 vs. control rats treated with feno.
15. Arthritic rats treated with fenofibrate have lower arthritis scores ($P < 0.05$) than the rats treated with vehicle from day 10. In addition, fenofibrate decreased arthritis-induced increase in spleen weight and hindpaw volume (Fig. 1, B and C). The anti-inflammatory effect of fenofibrate was also observed in TNFα gene expression in the liver, since fenofibrate administration decreased liver TNFα mRNA in arthritic rats (Fig. 1D).

PPARα expression in liver and gastrocnemius. Arthritis decreased the expression of PPARα mRNA to 40% in the liver and to 60% in the gastrocnemius (Fig. 2, A and B). The effect of fenofibrate on PPARα was different in liver than in gastrocnemius. In the gastrocnemius, fenofibrate induced a marked increase in PPARα mRNA in control and in arthritic rats. On the contrary, PPARα expression in the liver was not modified by this treatment.

Body weight gain, epididymal white adipose tissue, and gastrocnemius weight. On day 9 after adjuvant injection, arthritic rats treated with vehicle stopped gaining weight (Fig. 3A). This decrease in body weight gain was not due only to lower food intake, since pair-fed rats had higher body weight gain than arthritic rats treated with vehicle. Fenofibrate attenuated the decrease in food intake in arthritic rats between days 9 and 14, but food intake in this group was lower than in control rats, regardless of whether they were treated with fenofibrate (Fig. 3B). Body weight in the arthritic rats treated with fenofibrate was similar to that of the pair-fed rats and higher than that of arthritic rats treated with vehicle. In control rats, fenofibrate did not modify food intake or body weight gain.

Arthritis markedly decreased gastrocnemius and epididymal white adipose tissue weights to 63 and 46%, respectively, of the control rats (Fig. 3, C and D). The decrease in gastrocnemius weight does not seem to be secondary to arthritis-induced anorexia, since gastrocnemius weight, relative to body weight, in pair-fed rats was similar to that of control rats (Fig. 3C). In contrast, the decrease in white adipose tissue weight can be, in part, secondary to the decrease in food intake, since pair-fed rats had white adipose tissue values between those of control and arthritic rats (Fig. 3D). Fenofibrate attenuated the decrease in gastrocnemius, but not in white adipose weight in arthritic rats. In control rats, fenofibrate did not modify gastrocnemius or epididymal white adipose tissue weights.

Serum lipids. Both arthritis and fenofibrate administration decreased serum triglyceride levels (Table 1). In contrast, arthritis did not modify serum levels of lipoprotein HDL or LDL cholesterol (Table 1). As expected, fenofibrate decreased serum LDL cholesterol in control and in arthritic rats, whereas it increased HDL cholesterol in arthritic rats. Therefore, fenofibrate increased the HDL/LDL ratio in both control and arthritic rats.

Skeletal muscle histology. Figure 4A shows a cross-section of gastrocnemius, a muscle with two different areas, one mixed area with both slow-twitch and fast-twitch fibers and one area consisting of mostly fast-twitch fibers. Along with the decrease in gastrocnemius weight, gastrocnemius cross-section in arthritic rats was approximately one-half of the control rats (Fig. 4B). We measured fiber diameter to test whether or not the marked decrease in gastrocnemius mass might be caused by myofiber atrophy. Arthritis induced a decrease in both slow and fast mean fiber size (Fig. 4, C–E), where the decrease was higher in fast fiber. Fenofibrate treatment attenuated the loss in cross-sectional area and fast fiber size in arthritic rats, but the effect was significant only for fast fiber size.

Atrogene and Akt/FoxO3. The effect of arthritis and fenofibrate administration on atrogin-1 and MuRF1 expression in the gastrocnemius muscle is shown in Fig. 5, A and B. Expression of atrogene coincides with the different gastrocnemius size in the five groups. Arthritis increased the expression of atrogin-1 and MuRF1 ($P < 0.01$) in rats treated with vehicle. This effect is not due to the decrease in food intake, since pair-fed rats had atrogin-1 and MuRF1 levels similar to those of control rats. Fenofibrate administration to arthritic rats decreased atrogin-1 and MuRF1 mRNA to values similar to those of control or pair-fed rats. In control rats, fenofibrate treatment did not modify atrogin-1 or MuRF1 expression in the gastrocnemius muscle.

In contrast to atrogin-1 and MuRF1 expression, Akt signaling does not seem to be affected by arthritis or fenofibrate administration. Phospho-Akt and total Akt as well as phospho-Akt and total Akt ratio had similar values in all groups of rats (Fig. 6A). Similarly, arthritis did not modify phospho-FoxO3 or total FoxO3 or their ratio (Fig. 6B). Fenofibrate decreases...
phospho-FoxO3 protein in arthritic rats, but total FoxO3 or the phospho-FoxO3 and total FoxO3 ratio was not modified by fenofibrate administration.

**Myostatin.** We next evaluated whether the protective effect of fenofibrate is mediated by changes in myostatin (Fig. 7). Despite gastrocnemius atrophy, arthritis did not increase myostatin in the gastrocnemius muscle. Fenofibrate treatment decreased myostatin, both mRNA and protein, in the gastrocnemius of the arthritic rats. In control rats, fenofibrate administration also tended to decrease myostatin mRNA in the gastrocnemius, but this decrease was not significant. Pair-fed rats had similar myostatin levels than control rats treated with vehicle.

**Myogenic regulatory factors.** As reported previously (10), arthritis increased PCNA in the gastrocnemius of the rats treated with vehicle but not in those treated with fenofibrate (Fig. 8, A and B). Arthritis also induced upregulation of MyoD and myogenin expression in the gastrocnemius, whereas fenofibrate prevented an arthritis-induced increase in both myogenic regulatory factors (Fig. 8, C–F). In control rats treated with fenofibrate or in pair-fed rats, PCNA, MyoD, and myogenin levels were similar to those of the control rats treated with vehicle.

**DISCUSSION**

Our data indicate that chronic arthritis decreases PPARα expression, whereas treatment with fenofibrate has an anticaschetic action through downregulation of atrogin-1, MuRF1, and myostatin. The present data provide evidence that, in addition to its anti-inflammatory properties, fenofibrate partially prevents skeletal muscle wasting in chronic inflammation.

Arthritic rats have decreased PPARα expression in skeletal muscle and liver, whereas fenofibrate administration increased its expression in skeletal muscle. Inflammation-induced decrease in liver PPARα mRNA has been described previously following LPS administration (19, 20). In this inflammatory model, PPARα target genes involved in lipid metabolism are also decreased, suggesting that those responses play an important role in the alteration in lipid metabolism during acute-phase response (19). Downregulation of PPARα mRNA in skeletal muscle of arthritic rats does not seem to be due to the decrease in food intake, since pair-fed rats have higher PPARα expression in the gastrocnemius than arthritic rats. Furthermore, caloric restriction induces an increase in PPARα activity (13). Decrease in PPARα expression has also been reported recently in mus-
cachectic wasting induced by space flights (1). Similarly, in cachectic patients with chronic obstructive pulmonary disease, PPARα mRNA levels in skeletal muscle are increased and correlated negatively with inflammatory markers in plasma (47). These data, together with the fact that in arthritic rats fenofibrate attenuated the loss in gastrocnemius mass and PPARα, suggest that the decrease in PPARα expression, and possibly the genes involved in fatty acid oxidation, in skeletal muscle can thereby play an important role in muscle wasting.

As reported previously (42), fenofibrate decreased serum concentrations of triglycerides in control rats. This decrease has been explained as the result of increased fatty acid uptake and β-oxidation by the muscle. In the acute-phase response, adipose tissue lipolysis and reesterification of free fatty acid to triglycerides are increased, leading to an increase in serum triglyceride levels (28). On the contrary, arthritic rats have lower triglyceride levels than controls. One explanation for this difference can be the fact that hypertriglyceridemic response to LPS or cytokines is rapid, occurring within 2 h after administration (18). Accordingly, it is possible that chronic inflammation triggers lipid modifications that are different from those produced by the acute-phase response. In this sense, LPS increases white adipose tissue lipolysis (18), whereas arthritis decreases it (34). In arthritic rats, serum concentrations of HDL and LDL cholesterol were not modified. These data suggest that, in contrast to acute inflammation, chronic arthritis did not modify the lipoprotein profile in a significant way. Dyslipidemia has been reported in rheumatoid arthritis patients, but it has been attributed to the effect of steroid treatment and not to the illness (23).

As mentioned above, the anti-inflammatory effect of fenofibrate has been reported recently in rheumatoid arthritis patients, where fenofibrate ameliorated inflammation irrespective of improvement in dyslipidemia (23). The anti-inflammatory properties of fenofibrate have been also reported in other different inflammatory situations. Fenofibrate is also able to decrease TNFα in obese rats (4) as well as in patients with dyslipidemia (33). Fenofibrate antagonizes the inflammatory response, induced by LPS, through interference of Toll-like receptor 4 in vascular smooth muscle cells (27). Furthermore, it has also been reported that PPARα-deficient mice show an extended response to inflammation (45).

It has been reported that fenofibrate decreases fat mass, whereas it increases skeletal and lean mass and stimulates bone formation (54). However, we were unable to find an effect of fenofibrate in the gastrocnemius mass of control rats. Differences can be due to sex or to the length of treatment, since those females rats received fenofibrate for 4 mo. Nevertheless, fenofibrate administration had a stimulatory effect on gastrocnemius weight and on cross-sectional area in arthritic rats. Although our rats are juvenile, the inhibitory effect of arthritis on gastrocnemius weight is due not only to growth retardation but also to a progressive atrophy of muscle mass (10). Arthritis-induced decrease in gastrocnemius weight is associated with a reduction in cross-section size of both fiber types, fast and slow twitch. However, the atrophy is higher in fast-twitch fibers. These data are in accord with the fact that in arthritic rats muscle atrophy is higher in gastrocnemius, a mixed muscle consisting mainly of fast-twitch fibers, than in soleus, a slow-twitch muscle (31). They also suggest that slow-twitch muscle is more resistant to arthritis-induced atrophy than fast fibers. The fact that fast fibers are more susceptible to arthritis-induced atrophy does not seem to be due to less physical activity, since immobilization-induced muscle atrophy is more intense in soleus than in plantaris, a fast-twitch-type muscle (5). In accord with our data, in other states that induce cachexia, such as cancer, sepsis, and heart failure, fast fibers are more atrophied than slow fibers (3, 9, 55).

The increase in gastrocnemius weight in arthritic rats treated with fenofibrate was associated with normalization of expression of both atrogens atrogin-1 and MuRF1. This effect can be due to the anti-inflammatory action of fenofibrate, decreasing proinflammatory mediator, as we have observed with the cyclooxygenase-2 inhibitor meloxicam (25). It has been reported that PPARγ coactivator 1 (PGC-1) is an important factor in the skeletal muscle in delaying atrophy by inhibiting FoxO3-dependent transcription of atrogin-1 and MuRF1 (48). In addition, PGC-1 gene expression is decreased in atrophic muscle before the rise in proteolysis and weight loss. PGC-1 is also a coactivator of PPARα in the transcriptional control of mitochondrial fatty acid oxidation (56). PPARα is induced by PGC-1 (36), and some effects of PGC-1 are secondary to the action of PGC-1 on the PPARα pathway (29). Therefore, it
could be possible that fenofibrate directly inhibits atrogin-1 and MuRF1 upregulation by interfering with the FoxO3 pathway. However, this does not seem to be the case, since arthritis did not modify the Akt/FoxO3 signaling. The lack of modifications in Akt signaling in the gastrocnemius of arthritic rats is in accord with the increased expression of IGF-I and IGF-I receptor in the gastrocnemius of these rats (10). Similarly, it has been reported that in other chronic inflammatory illnesses such as chronic obstructive pulmonary disease, muscle atrophy is not due to a decrease in Akt signaling (16). Although not investigated in this study, we cannot rule out that FoxO1 or FoxO4 may have an important role in the induction of muscle atrophy in arthritic rats. In this sense, it has been reported that TNF induction of atrogin-1 is by activation of FoxO4 but is independent of the Akt pathway (40).

Myostatin has been postulated as a powerful negative regulator of muscle mass (46). In addition, myostatin contributes to muscle atrophy in food deprivation (2). However, as we have reported previously (10), arthritis-induced gastrocnemius atrophy does not seem to be associated with an increase in myostatin expression. Similarly, myostatin is not upregulated in sepsis (51). Myostatin downregulation can probably contribute to the anticachetic effect of fenofibrate in arthritic rats. Fenofibrate-induced increase in muscle fiber size in arthritic rats can be due to myostatin decrease, since myostatin inactivation results in muscle fiber hypertrophy (58). In addition, myostatin-deficient mice exhibit markedly increased muscle mass composed mainly of fast fibers (38). These data indicate that the higher stimulatory effect of fenofibrate on fast fiber size in arthritic rats can be due to the decrease in myostatin. Myostatin downregulation can also contribute to the decrease in atrogin-1 and MuRF1, since this protein has been reported to induce both atrogenes (37). Although myostatin inactivation has been reported to increase PPARα expression (53), the
mechanism by which fenofibrate administration decreases myostatin is not known. This effect can be directly exerted on skeletal muscle cell, since myostatin promoter sequence contains putative muscle growth response elements for PPAR (32). Nevertheless, it is also possible that fenofibrate modifies myostatin expression as a result of its metabolic actions.

As we reported previously (10), despite gastrocnemius wasting, there was an increase in PCNA, MyoD, and myogenin in arthritic rats. A relationship between inflammation and muscle regeneration has been reported (35, 41). In this sense, the proinflammatory cytokines TNFα and interleukin-6 have been reported to promote myogenesis (8, 12). The fact that fenofibrate prevented an arthritis-induced increase in these myogenic regulatory factors can be due to its anti-inflammatory effect. As mentioned above, EPA, a natural ligand of PPARs, prevents arthritis-induced increases in atrogin-1 and MuRF1 (11). However, PCNA, MyoD, and myogenin expression remained elevated in arthritic rats treated with EPA. Furthermore, in control rats, EPA treatment increased PCNA and MyoD (11). These data suggest that some of the effects of EPA on skeletal muscle are not mediated through PPAR activation.

In conclusion, the present study suggests that arthritis-induced PPARα downregulation in skeletal muscle plays a role in muscle wasting. Furthermore, PPARα activation by fenofibrate has an anticachetic effect, decreasing atrogin-1, MuRF1, and myostatin expression in the skeletal muscle. These data provide a new mechanism related to regulating muscle mass and energy balance and inflammation. Our observations suggest that fenofibrate treatment could be useful in reducing arthritis symptoms and in preserving muscle mass in rheumatoid arthritis patients having dyslipidemia.

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DISCLOSURES

The authors have nothing to disclose.

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