Eicosapentaenoic acid attenuates arthritis-induced muscle wasting acting on atrogin-1 and on myogenic regulatory factors

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Castillero E, Martín AI, López-Menduiña M, Villanúa MA, López-Calderón A. Eicosapentaenoic acid attenuates arthritis-induced muscle wasting acting on atrogin-1 and on myogenic regulatory factors. Am J Physiol Regul Integr Comp Physiol 297: R1322–R1331, 2009. First published September 9, 2009; doi:10.1152/ajpregu.00388.2009.—Eicosapentaenoic acid (EPA) is an omega-3 polyunsaturated fatty acid that has anti-inflammatory and anticaichetic actions. The aim of the work was to elucidate whether EPA administration is able to prevent an arthritis-induced decrease in body weight and muscle wasting in rats. Arthritis was induced by intradermal injection of Freund’s adjuvant; 3 days later, nine rats received 1 g/kg EPA or coconut oil daily. All rats were killed 15 days after adjuvant injection. EPA administration decreased the external signs of arthritis and paw volume as well as liver TNF-α mRNA. EPA did not modify arthritis-induced decrease in food intake or body weight gain. However, EPA treatment prevented arthritis-induced increase in muscle TNF-α and atrogin-1, whereas it attenuated the decrease in gastrocnemius weight and the increase in MuRF1 mRNA. Arthritis not only decreased myogenic regulatory factors but also increased PCNA, MyoD, and myogenin mRNA in the gastrocnemius. Western blot analysis showed that changes in protein content followed the pattern seen with mRNA. In the control rats, EPA administration increased PCNA and MyoD mRNA and protein. In arthritic rats, EPA did not modify the stimulatory effect of arthritis on these myogenic regulatory factors. The results suggest that in experimental arthritis, in addition to its anti-inflammatory effect, EPA treatment attenuates muscle wasting by decreasing atrogin-1 and MuRF1 gene expression and increasing the transcription factors that regulate myogenesis.

adjuvant-induced arthritis; ubiquitin-proteasome system; MyoD; myogenin; proliferating cell nuclear antigen

CHRONIC INFLAMMATORY DISEASES such as cancer, sepsis, and rheumatoid arthritis are associated with a decrease in body weight, skeletal muscle atrophy, and cachexia. Cachexia is a complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat mass (12). Adjuvant-induced arthritis is a widely used experimental model because in many respects it mimics rheumatoid arthritis in humans (45). Arthritis can be induced in rats by an intradermal injection of Freund’s adjuvant (heat-killed Mycobacterium butyricum). On days 10 and 11 after adjuvant injection, rats develop chronic inflammation and polyarthritis that lead to a marked decrease in body weight and cachexia (37) by a dramatic loss of adipose and skeletal muscle mass (8, 31). Cachexia also has been reported in rheumatoid arthritis patients, adversely affecting morbidity and mortality (36). In rheumatoid arthritis patients, weight is lost equally from adipose tissue and muscle and is not secondary to a decrease in caloric intake, but rather to an increase in resting energy expenditure (2). Similarly, a decrease in the relative skeletal muscle mass is observed in arthritic rats but not in pair-fed rats (8).

Muscle wasting in arthritic rats is associated with an increase in the gene expression of two genes of the ubiquitin-proteasome system, MuRF1 (muscle ring finger 1) and atrogin-1 (14). These genes are known as “atrogenes,” since they are upregulated in several conditions that induce muscle wasting, such as cancer, sepsis, diabetes, and fasting (27). In addition, mice lacking these genes are resistant to denervation-induced muscle wasting (5). For that reason, these genes serve as early markers of skeletal muscle atrophy, aiding in the diagnosis of muscle disease. In arthritic rats, the upregulation of atrogenes is specific to the skeletal muscle, since it does not occur in the cardiac muscle; accordingly, there is no wasting in the cardiac muscle in chronic arthritis (14).

It is well known that omega-3 polyunsaturated fatty acids (PUFA) have beneficial effects on cardiovascular health and on inflammatory diseases. Eicosapentaenoic acid (EPA) is a PUFA that is essential for normal growth and development, since it is part of the cellular membranes. EPA has anti-inflammatory actions in both human and experimental animals. A diet rich in fish, in which EPA is the major component, is able to ameliorate autoimmune diseases (33). There are several clinical studies that show benefits from fish oil in patients with rheumatoid arthritis (23). Among the lipid mediators of the fish oil, EPA has been shown to reduce joint stiffness in rheumatoid arthritis patients (46).

EPA competes with arachidonic acid for incorporation in the cell membrane phospholipid, as a substrate of cyclooxygenase (COX)-2, leading to a decrease in PGE2 synthesis. We recently reported that COX-2 inhibition by meloxicam administration has an important anticaichetic effect in arthritic rats (15) by preventing arthritis-induced increase in MuRF1 and atrogin-1 gene expression in the skeletal muscle. However, anti-inflammatory treatment with COX-2 inhibitors has been demonstrated to have several side effects on the cardiovascular system (16).

In addition to its anti-inflammatory effect, the anticaichetic effect of fish oil treatment in cancer cachexia is well known. EPA administration is able to prevent cancer-induced decrease in body weight gain and skeletal muscle wasting in both human (13) and experimental animals (49). The beneficial effect of EPA on skeletal muscle wasting is secondary to a decrease in muscle protein degradation by preventing the activation of the ubiquitin-proteasome pathway. This mechanism has been reported in cancer (41), hyperthermia (42), and fasting (48).
Furthermore, only one day of administration of EPA is able to prevent sepsis-induced muscle proteolysis in mice (25).

Taking into account the EPA anti-inflammatory effect in arthritis and the observed muscle atrophy in chronic arthritis, the purpose of this study was to examine whether one of the beneficial effects of EPA on chronic arthritis could be a reduction in skeletal muscle atrophy. For this purpose, expression of atrogin-1 and MuRF1 in the gastrocnemius muscle of arthritic rats treated with EPA was analyzed. Proliferation and differentiation of muscular precursor cells, or satellite cells, into mature muscular cells depends on hormones and growth factors such as PCNA, MyoD, and myogenin. Because adjuvant-induced arthritis also increases the expression of the myogenic regulatory factors PCNA, MyoD, and myogenin (8), their response to EPA administration was also analyzed.

**MATERIAL AND METHODS**

**Animals.** Arthritic and control male Wistar rats (100–125 g, 5 wk old) were purchased from Charles River Laboratories (Barcelona, Spain). Arthritis was induced in the rats by an intradermal injection of 4 mg of heat-inactivated *M. butyricum* in the right paw under isoflurane anesthesia. Control animals were injected with vehicle (0.1 ml of paraffin oil). After arrival (day 3 after adjuvant injection), rats were housed three or four per cage and maintained under standardized conditions of temperature (20–22°C) and light (lights on from 0730 to 1930 h). Water and standard chow (A04; Panlab, Barcelona, Spain) were provided ad libitum. The procedures followed 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 3 after adjuvant injection, both control rats and rats injected with adjuvant were randomly divided into two groups, with 9 animals in each treatment group. The first group received, at a dosage of 1 g/kg body wt daily by oral gavage, highly purified ethyl ester of eicosapentaenoic acid (E-EPA) containing 90% EPA and 0.02% vitamin D3 (Oy Bio-Vita Ab, Espoo, Finland). The other group received 1 g/kg body wt of coconut oil to ensure isocaloric intake. A pair-fed group was also included, since arthritis and reverse primers in a reaction volume of 25.5 μl. Primers for real-time PCR (Table 1) were obtained from Roche (Madrid, Spain) using the EXIQON Universal Probe Library (atrogin-1, myostatin, PCNA, and myogenin) or from previously published sequences of MuRF-1 (11), MyoD (19), and 18S (6). 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 5 s, and 72°C extension steps for 30 s. Results were expressed relatively to the control animals treated with coconut oil, where the relative mRNA abundance was arbitrarily set to 1, using the cycle threshold 2(ΔΔC T) method (29) with 18S as reference gene. PCR products were separated using agarose gel electrophoresis to confirm the product presence and size.

**Immunoblot.** Muscle samples were homogenized in lysis buffer (10 μl/mg) with protease inhibitor cocktail (Sigma-Aldrich, Madrid, Spain). The homogenate was later centrifuged at 13,000 rpm at 4°C for 30 min to remove tissue debris. Protein concentration was determined using the Bradford protein assay with bovine serum albumin as standard. The protein extract was boiled for 5 min with a 1:1 volume of Laemmli loading buffer. Proteins (50 μg) were resolved by electrophoresis on 14% polyacrylamide gels under reducing conditions and then transferred onto nitrocellulose membranes that were blocked by incubation in 5% nonfat dry milk and 0.1% Tween (Sigma-Aldrich) in Tris-buffered saline. Membranes were probed overnight at 4°C sequentially with antibodies against myogenin, PCNA, myostatin, MyoD (Santa Cruz Biotechnology, Santa Cruz, CA), and α-tubu-

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer (5’ to 3’)</th>
<th>Reverse Primer (5’ to 3’)</th>
<th>Product Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>18S</td>
<td>GCTGCGATGCGGCTTTCTCTTA</td>
<td>GCTTCTGTTTATCGGAATTACC</td>
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<tr>
<td>TNF-α</td>
<td>GCCACGAGCTTCCTGTGCTT</td>
<td>GCTTCGGCCATGGACCTGT</td>
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<td>Atrogin-1</td>
<td>GAAGCAGAAAAACCGAAATCTCAAGTA</td>
<td>GTCCTTTAATGACTCCTTTTTGAAA</td>
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<td>MuRF-1</td>
<td>TGTCCTGGAGGCGTGTTGGGCG</td>
<td>ATGCCGGTACATGCTACCT</td>
<td>58</td>
</tr>
<tr>
<td>Myostatin</td>
<td>TGCGGGATGTGCTTGTTAG</td>
<td>TGTTACCTTTGACCTTAAAAGGGATT</td>
<td>76</td>
</tr>
<tr>
<td>PCNA</td>
<td>TGAAATTTCGGGAGAAACCTACT</td>
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<tr>
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<td>AGCACTCTGGTAATCGGATGCG</td>
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</tr>
<tr>
<td>Myogenin</td>
<td>GGTTGCGTGGATGCTCCTCTGA</td>
<td>GGAGTTGAGATTCCATCGCGAGG</td>
<td>94</td>
</tr>
</tbody>
</table>
lin (Sigma-Aldrich) with stripping of membranes before each new antibody. Membranes were then incubated for 90 min in the appropriate horseradish peroxidase-conjugated secondary antibody (anti-mouse IgG, Amersham Biosciences, Little Chalfont, UK; anti-rabbit IgG, Bio-Rad, Madrid, Spain), and peroxidase activity was detected using enhanced chemiluminescent reagent (Amersham Biosciences). Band intensities were quantified by densitometry using Gene Tools Analysis software. The density of the protein band in each lane is expressed as the percentage of the mean density of control rat values after loading normalization using α-tubulin.

Statistical analysis. Results were compared using the statistics program Statgraphics Plus for Windows. Normal distribution of data was assessed using a Shapiro-Wilks test. Continuous variables are means ± SE and were tested with analysis of variance (ANOVA); post hoc comparisons were made using the least significant difference multiple range test. Data that were not normally distributed (TNF, atrogin-1, and MuRF1 mRNA in the gastrocnemius) were analyzed using the Mann-Whitney (Wilcoxon) W test; those data are presented as scatter plots with median lines. Arthritis score index was analyzed using an unpaired Student’s t-test. Statistical significance was set at $P < 0.05$.

RESULTS

As shown in Fig. 1A, on day 10 after adjuvant injection, the arthritis score increased in the arthritic rats fed with coconut oil than in rats treated with EPA (P < 0.05). EPA administration decreased the paw volume in arthritic rats (P < 0.01) but not control rats. Arthritis increased liver TNFα mRNA in rats treated with coconut oil (P < 0.01), whereas it had no effect on rats treated with EPA. Similarly, arthritis increased TNFα mRNA (P < 0.01) in the gastrocnemius of the rats treated with coconut oil but not in the rats treated with EPA. AA, arthritic rats; PF, pair-fed rats; coco, coconut oil. Data in A–C are means ± SE; data in D are presented as scatter plots with medians. *P < 0.05; **P < 0.01 vs. control rats treated with coconut oil. ##P < 0.01 vs. arthritic rats treated with coconut oil. +P < 0.05 vs. control rats treated with EPA.
oil, reaching its highest value on day 15. In the arthritic rats that received EPA, the increase in the arthritis scores was lower than in the rats treated with coconut oil (P < 0.01). In the group treated with coconut oil, all rats had arthritis in other than the right hind paw. In contrast, in the group of rats treated with EPA, two rats had arthritis only in the right hind paw, two rats had arthritis in just other than the injected paw (but the arthritis score decreased from day 12 to day 15), and the other five rats had arthritis in the four limbs. The anti-inflammatory effect of EPA also was evident in the volume of the left hind paw (Fig. 1B). The arthritic rats treated with coconut oil had increased paw volume (P < 0.01), whereas the arthritic rats treated with EPA had lower paw volume than the arthritic rats treated with coconut oil (P < 0.01), but this was higher than that observed in the control or pair-fed rats (P < 0.05).

In the rats treated with coconut oil, arthritis increased TNF-α gene expression in the liver (P < 0.01; Fig. 1, C and D) and in the gastrocnemius muscle (P < 0.01). However, the arthritic rats treated with EPA had TNF-α mRNA values similar to those of the control or pair-fed rats in both the liver and the gastrocnemius.

The evolution of body weight is shown in Fig. 2A. Arthritis decreased body weight gain; this difference was statistically significant from day 6 after adjuvant injection (P < 0.01). From day 10 to day 15, arthritic rats did not gain body weight. The decrease in body weight gain is due not only to lower food intake but also to inflammation, since pair-fed rats had higher body weight gain than the arthritic rats. Arthritis decreased food intake (P < 0.01), whereas EPA administration did not modify food intake in either control or arthritic rats (Fig. 2B). There was a decrease in the relative gastrocnemius weight (P < 0.01) in the arthritic rats treated with coconut oil but not in pair-fed rats. EPA administration increased the relative gastrocnemius weight in arthritic rats (P < 0.01; Fig. 2C).

As expected, the arthritic rats treated with coconut oil increased the expression of both atrogenes MuRF1 and atro-

Fig. 2. Evolution of body weight gain (A), food intake between days 4 and 15 (B), and relative gastrocnemius weight (C) in control, arthritic, or pair-fed rats treated with 1 g/kg coconut oil or 1 g/kg EPA. Arthritis decreased food intake (P < 0.01) and the relative gastrocnemius weight (P < 0.01). EPA administration did not modify food intake but increased gastrocnemius weight in arthritic rats (P < 0.01). Results are means ± SE for n = 3 cages and n = 7–9 rats per group. **P < 0.01 vs. control rats treated with coconut oil. #P < 0.05; ##P < 0.01 vs. arthritic rats treated with coconut oil. ++P < 0.01 vs. control rats treated with EPA.
Atrogin-1 in the gastrocnemius muscle \((P < 0.01; \text{Fig. 3, } A \text{ and } B)\). EPA administration prevented the effect of arthritis on atrogin-1 mRNA and attenuated the effect of arthritis on MuRF1 mRNA in the gastrocnemius. The expression of MuRF1 and atrogin-1 in the gastrocnemius of the pair-fed rats was similar to that of the control rats.

Figure 4 shows myostatin in gastrocnemius muscle of the five experimental groups. EPA administration tended to decrease myostatin in the gastrocnemius of the arthritic rats, but there was no significant difference in myostatin mRNA or myostatin protein between the groups.

As previously reported \((8)\), arthritis induced an increase in PCNA mRNA \((P < 0.01)\) and PCNA protein \((P < 0.05)\) in the rats that received coconut oil (Fig. 5, A and B). EPA adminis-
tration tended to elevate both mRNA and protein of PCNA in control rats to values similar to those observed in arthritic rats, although this increase was not significant. The arthritic rats treated with EPA had similar PCNA mRNA and protein compared with the arthritic rats treated with coconut oil.

The effect of EPA administration on MyoD in the gastrocnemius is shown in Fig. 6, A and B. EPA administration increased MyoD mRNA and protein in control rats, but only the increase in mRNA was significant (P < 0.05). Arthritis increased MyoD mRNA and protein (P < 0.05), with values similar to those of the arthritic rats treated with coconut oil or with EPA (Fig. 6, A and B).

EPA administration did not modify myogenin mRNA or protein in the gastrocnemius of the control rats (Fig. 7, A and B). Arthritis increased myogenin mRNA and protein (P < 0.01) in both groups of arthritic rats treated with coconut oil or with EPA.

The effects of arthritis on gene expression and the protein of the different muscle regulatory factors (myostatin, PCNA, MyoD, and myogenin) were not secondary to the decrease in food intake, since pair-fed rats had values similar to those observed in control rats treated with coconut oil (Figs. 4–7).

DISCUSSION

Our data show that EPA administration to arthritic rats has an anti-inflammatory effect and attenuates skeletal muscle wasting. The protective effect of EPA on the gastrocnemius muscle is due not only to a decrease in atrogene expression but also to a stimulatory effect on myogenic regulatory factors.

The effects of EPA administration on the development of arthritis are in agreement with previous reports showing, in several models of arthritis, that fish oil administration has an anti-inflammatory effect (28, 46). A beneficial effect of fish oil supplements in rheumatoid arthritis patients also has been reported (22). Furthermore, EPA suppresses the “in vitro” proliferation of synoviocytes from rheumatoid arthritis patients (18). EPA also is able to reduce the expression of COX-2 and inflammatory cytokines, as well as cartilage-degrading protein, in chondrocyte cultures (51). The anti-inflammatory effect of EPA in the arthritic rats can be related to its inhibitory action on TNF-α expression. The inhibitory effect of EPA on TNF-α synthesis and release, as well as on TNF-α-induced activation of the NF-κB pathway, has been observed in several cell types after inflammatory stimuli (26, 52). It has been proposed that the anti-inflammatory effect of EPA is exerted through membrane phospholipids, becoming incorporated into the cell membrane instead of arachidonic acid and modifying eicosanoids synthesis by reducing the formation of proinflammatory eicosanoids (e.g., PGE2) (3). On the other hand, EPA can form several potent anti-inflammatory lipid mediators such as resolvin E1 (RvE1), which counteracts the effect of TNF-α and inhibits NF-κB activation (for review, see Ref. 39). RvE1 plays a role in the resolution of the inflammatory response and reduces inflammation to a lower extent in several animal models of inflammatory diseases (20, 50).

As previously reported in arthritic rats (46), EPA treatment decreased footpad inflammation without modifying food intake or body weight in arthritic rats. In contrast, in cancer cachexia, EPA preserves body weight (38). This difference could be due to the fact that although cancer and arthritis induce muscle...
wasting and fat mass loss, these mechanisms do not seem to be identical in both illnesses. Muscle wasting is associated with an increase in myostatin in cancer (10), whereas no modification in myostatin was observed in the skeletal muscle of arthritic rats. Furthermore, fat mass loss in arthritis is associated with a decrease in adipogenesis rather than an increase in lipolysis (31). On the other hand, in cancer, lipolysis is induced by an increase in zinc-α2-glycoprotein (ZAG), which plays an important role in loss of fat mass (4), whereas ZAG expression is not modified in arthritic rats (31). In cancer cachexia, EPA

**Fig. 6.** MyoD mRNA (A), MyoD protein (B), and representative immunoblots (C) in the gastrocnemius of control, arthritic, and pair-fed rats treated with 1 g/kg coconut oil or 1 g/kg EPA from day 3 to day 15 after adjuvant injection. PCNA mRNA was measured by real-time PCR, and results are expressed relative to those of control animals treated with coconut oil, by analyzing the CT numbers corrected by CT readings of corresponding internal 18S mRNA controls. MyoD protein was measured by Western blotting, quantified, normalized against α-tubulin, and expressed as a percentage of the value for control rats treated with coconut oil. Arthritis increased MyoD mRNA (P < 0.05) and MyoD protein (P < 0.05) in rats treated with coconut oil. EPA increased MyoD mRNA (P < 0.05). Data are means ± SE (n = 7–9 rats). *P < 0.05 vs. control rats treated with coconut oil.

**Fig. 7.** Myogenin mRNA (A), myogenin protein (B), and representative immunoblots (C) in the gastrocnemius of control, arthritic, and pair-fed rats treated with 1 g/kg coconut oil or 1 g/kg EPA from day 3 to day 15 after adjuvant injection. Myogenin mRNA was measured by real-time PCR, and results are expressed relative to those of control animals treated with coconut oil, by analyzing the CT numbers corrected by CT readings of corresponding internal 18S mRNA controls. Myogenin protein was measured by Western blotting, quantified, normalized against α-tubulin, and expressed as a percentage of the value for control rats treated with coconut oil. Arthritis increased myogenin mRNA (P < 0.01) and myogenin protein (P < 0.01) in rats treated with coconut oil or EPA. Data are means ± SE (n = 7–9 rats). **P < 0.01 vs. control rats treated with coconut oil. +P < 0.05; ++P < 0.01 vs. control rats treated with EPA.
prevents adipose tissue loss by downregulation of ZAG expression through interference with glucocorticoid signaling (38).

Despite the fact that EPA does not have an effect on body weight gain, EPA treatment increased gastrocnemius weight in arthritic rats. This effect can be explained by the fact that EPA administration prevented atrogin-1 and attenuated MuRF1 arthritis-induced increase in the gastrocnemius. To our knowledge, the effect of EPA on atrogin-1 or MuRF1 expression has not been previously reported. However, it has been reported that EPA is able to prevent the upregulation of other components of the ubiquitin-proteasome system. In this sense, EPA attenuates muscle protein degradation in cancer and sepsis by preventing the increase in both gene expression and protein of the α- and β-subunits of the 20S proteasome, as well as the functional activity of the proteasome (25, 49). Taking into account that the ubiquitin-proteasome proteolytic system is the main contributor to muscle wasting in cachexia (27), the beneficial action of EPA on muscle loss could be due to its action on the ubiquitin-proteasome pathway. Together these data suggest that EPA decreases the activity of the ubiquitin-proteasome pathways induced by chronic inflammation. The inhibitory effect of EPA on skeletal muscle proteolysis can be exerted directly on the muscular cell, since EPA is able to prevent hyperthermia-induced proteolysis by the ubiquitin-proteasome in myotube cultures (42).

The muscular wasting observed in the arthritic rats and the beneficial effect of EPA are independent of food intake, since as previously reported (8), the decrease in the relative gastrocnemius weight is not observed in pair-fed rats, and EPA administration increased gastrocnemius weight without modifying food intake in arthritic rats.

Myostatin is a negative regulator of muscle growth that increases in situations of muscle atrophy, such as cancer cachexia (10). However, myostatin is not modified by chronic arthritis or EPA administration. Similarly, myostatin was not modified 15 days after immobilization, although a reduction in quadriceps lean mass was observed (24).

As we previously reported (8), despite gastrocnemius wasting, there was an increase in PCNA, MyoD, and myogenin in arthritic rats. These data indicate that in arthritic rats, muscle repair/regeneration coexists with the activation of the ubiquitin-proteasome pathway. Similarly, short bouts of passive stretching are able to increase the gene expression of factors associated with muscle growth (MyoD) and atrophy (atrogin-1) (35). The factors responsible for the upregulation of these myogenic regulatory factors in the gastrocnemius of arthritic rats are unknown. However, there is evidence that suggests a relationship between inflammation and muscle regeneration (32, 44). The proinflammatory cytokines TNF and IL-6 have been reported to promote myogenesis (7, 9). Furthermore, we have observed that administration of the nonsteroid anti-inflammatory drug meloxicam to arthritic rats prevents the increased expression of TNF, atrogin-1, and MuRF1 in the gastrocnemius (15). It was recently reported that another COX-2 inhibitor, NS-398, decreases muscle hypertrophy after synergist ablation (34).

EPA administration prevented arthritis-induced increases in atrogin-1 and MuRF1, whereas PCNA, MyoD, and myogenin expression remained elevated in arthritic rats treated with EPA. Furthermore, in control rats, EPA treatment increased PCNA and MyoD. These data suggest that, in addition to the inhibitory effect on atrogin-1 and MuRF1, part of the beneficial effect of EPA on the gastrocnemius mass can be mediated by stimulating myogenic regulatory factors. To our knowledge, the effect of EPA on the regulatory myogenic factor in vivo has not been described previously. It has been reported that EPA is able to prevent the inhibitory effect of high levels of TNF-α on C2C12 myotube myogenesis by increasing myotube diameter and myoglobin high chain expression (30).

In the muscle cell in vitro, EPA directly modulates lipid and glucose metabolism, promoting increased glucose uptake and metabolism (1). A similar effect of EPA was recently described in diabetic myotubes, where EPA also improved insulin resistance and fatty acid handling in type 2 diabetes skeletal muscle (47). Proteolysis-inducing factor (PIF), secreted by tumors that induce cachexia, decreases glucose uptake by myoblast and induces proteolysis, which are the two effects attenuated by EPA (21).

Perspectives and Significance

Rheumatoid cachexia is an important contributor in increasing morbidity and premature mortality in rheumatoid arthritis patients. Adjuvant-induced arthritis is a well-established model of rheumatoid arthritis that is associated with cachexia and muscle wasting secondarily to an increase in the activity of the ubiquitin-proteasome proteolytic pathway. The data presented in this article demonstrate that administration of the omega-3 polyunsaturated fatty acid EPA to arthritic rats decreases the external signs of inflammation and TNF-α expression in the gastrocnemius muscle, whereas it increases gastrocnemius weight. EPA treatment ameliorates skeletal muscle wasting by preventing arthritis-induced increase in the expression of the E3 ubiquitin-ligating enzymes atrogin-1 and MuRF1 in the gastrocnemius. In addition, EPA treatment also is able to increase the transcription factors that regulate myogenesis, such as PCNA and MyoD. Our observations suggest that EPA treatment could be used therapeutically to reduce symptoms of arthritis and to preserve muscle mass in rheumatoid arthritis.

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EPA PREVENTS MUSCLE WASTING IN ARTHRITIC RATS

R1330


