Comparison of the effects of the n-3 polyunsaturated fatty acid eicosapentaenoic and fenofibrate on the inhibitory effect of arthritis on IGF1

Estíbaliz Castellero, María López-Menduiña, Ana Isabel Martín, María Ángeles Villanúa and Asunción López-Calderón

Department of Physiology, Faculty of Medicine, Complutense University of Madrid, 28040 Madrid, Spain

(Correspondence should be addressed to A López-Calderón; Email: alc@med.ucm.es)

Abstract

Adjuvant-induced arthritis is a chronic inflammatory illness that induces muscle wasting and decreases circulating IGF1. Eicosapentaenoic acid (EPA) and fenofibrate, a peroxisome proliferator-activated receptors α agonist, have anti-inflammatory actions and ameliorate muscle wasting in arthritic rats. The aim of this work was to elucidate whether EPA and fenofibrate administration are able to prevent the effect of arthritis on the IGF1–IGFBP system. On day 4 after adjuvant injection control, arthritic rats were gavaged with EPA (1 g/kg) or fenofibrate (300 mg/kg) until day 15 when all rats were killed. Arthritis decreased body weight gain, serum IGF1, and liver *Igf1* mRNA, whereas it increased gastrocnemius *Igf1* mRNA. EPA, but not fenofibrate,

administration prevented arthritis-induced decrease in serum IGF1 and liver *Igf1* mRNA. In the rats treated with EPA arthritis increased *Igf1* mRNA in the gastrocnemius. Fenofibrate treatment decreased IGF1 and *Igf1* mRNA in the liver and gastrocnemius. In arthritic rats, fenofibrate increased body weight gain and decreased gastrocnemius *Igf1* and *Igf1* mRNA. These data suggest that the mechanisms through which EPA and fenofibrate act on the IGF1 system and ameliorate muscle wasting in arthritic rats are different. EPA administration increased circulating levels of IGF1, whereas fenofibrate decreased the *Igf1* and *Igf1* in the gastrocnemius muscle.

Journal of Endocrinology (2011) **210**, 361–368

Introduction

Chronic inflammation is often associated with skeletal muscle wasting and cachexia (Evans *et al.* 2008). Inflammatory cachexia has been reported in chronic illnesses such as cancer, heart or renal failure, Crohn's disease, sepsis, and rheumatoid arthritis. Cachexia can also induce a decrease in body weight that is associated with muscle atrophy and sometimes with fat mass depletion. The increased release of cytokines and other proinflammatory mediators plays a crucial role in inflammatory cachexia (Morley *et al.* 2006). However, inflammation induces deep modifications in the neuroendocrine system that might also contribute to a decrease in body weight, muscle wasting, and cachexia (Morley *et al.* 2006). These modifications include an increase in the release of glucocorticoids with a decrease in anabolic hormones such as androgens and insulin-like growth factor 1 (IGF1; Soto *et al.* 1998).

Adjuvant-induced arthritis is an experimental model of rheumatoid arthritis that is associated with a decrease in body weight and muscle wasting (Castellero *et al.* 2009a). Arthritic rats have low serum IGF1 levels that are inversely related to body weight gain (López-Calderón *et al.* 1999), whereas

chronic GH administration to arthritic rats increases body weight and serum IGF1 levels (Ibañez de Cáceres *et al.* 2000). Similarly, GH administration to juvenile RA patients increases growth (Touati *et al.* 1998). Furthermore, exogenous IGF1 administration to arthritic rats increases body and skeletal muscle weights (López-Menduiña *et al.* 2010). All these data indicate that arthritis-induced decrease in circulating IGF1 is an important contributor to the decrease in body weight and skeletal muscle wasting induced by experimental arthritis.

Eicosapentaenoic acid (EPA) is an n-3 polyunsaturated fatty acid that is essential for normal growth and is present in large amounts in fish oil. It is well known that EPA has an anti-inflammatory effect decreasing proinflammatory cytokines (Tashiro *et al.* 1998, Zhao *et al.* 2004) and both cyclooxygenase-2 activity and expression (Hurst *et al.* 2009). Furthermore, EPA is able to decrease joint inflammation in rheumatoid arthritis patients (James & Cleland 1997, Simopoulos 2002) and in arthritic rats (Leslie *et al.* 1985, Volker *et al.* 2000, Castellero *et al.* 2009b). In addition to its anti-inflammatory effect, EPA ameliorates arthritis-induced skeletal muscle wasting by decreasing *atrogin-1* and *Murf-1* gene expression and also increasing the transcription factors

that regulate myogenesis (Castillero *et al.* 2009a). Taking into account that the beneficial effect of IGF1 treatment on skeletal muscle in arthritic rats is also associated with decreased *atrogin-1* and increased myogenic regulatory factors (López-Menduiña *et al.* 2010), it is possible that EPA administration can prevent arthritis-induced inhibition of IGF1.

EPA has several mechanisms of action, and one of these is through the activation of peroxisome proliferator-activated receptors (PPARs; Forman *et al.* 1997). PPARs are nuclear transcription factors that regulate genes involved in the control of metabolism and inflammatory responses (Michalik & Wahli 2008). In hepatocytes, EPA has been reported to activate PPAR α (Pawer & Jump 2003) and upregulate *Ppara* gene expression (Liu *et al.* 2011). Fenofibrate, a PPAR α ligand, inhibits cytokine production from rheumatoid synovial fibroblasts and inhibits the development of adjuvant-induced arthritis in female Lewis rats (Okamoto *et al.* 2005). In arthritic rats, we have reported that fenofibrate administration increases body weight and decreases skeletal muscle atrophy through inhibition of *atrogin-1*, *Murf-1*, and *myostatin* expression in skeletal muscle (Castillero *et al.* 2011). These data indicate that EPA and fenofibrate have comparable effects on skeletal muscle in arthritic rats. To clarify whether the effect of EPA can be mediated by PPAR α activation, we have compared the response of the IGF-IGFBPs system to experimental arthritis in rats fed with either EPA or the PPAR α activator fenofibrate.

Materials and Methods

Animals

Arthritic and control male Wistar rats weighing 150 g were purchased from Charles River (Barcelona, Spain). Arthritis was induced in the rats by a s.c. injection of 4 mg heat-inactivated *Mycobacterium butyricum* in the right hind paw, under isoflurane anesthesia (Castillero *et al.* 2009a). Control animals were injected with vehicle (0.1 ml paraffin oil). After arrival (day 3 after adjuvant injection), rats were housed 3 per cage, and maintained under standardized conditions of temperature (20–22 °C) and light (lights on from 0730 to 1930 h). Assessment of arthritis was performed by measuring the arthritis index of each animal, which was clinically scored by grading each paw from 0 to 4. Grading was determined as: 0, no erythema or swelling; 1, slight erythema or swelling of one or more digits; 2, swelling of paw; 3, swelling of entire paw and ankle; and 4, ankylosis, incapacity to bend the ankle. The severity score was the sum of the clinical scores of each limb, the maximum value being 16 (Tanaka *et al.* 1996). The procedures followed the guidelines recommended by the EU for the care and use of laboratory animals, and were approved by the Complutense University animal care committee.

Treatments

EPA 18 control and 18 arthritic rats were divided into two groups on day 4 after adjuvant injection. The first group received a daily dose of 1 g/kg BW highly purified ethyl ester of EPA (E-EPA) containing 90% EPA and 0.02% vitamin D₃ (Oy Bio-Vita Ab, Espoo, Finland) by oral gavage. The other group received 1 g/kg BW of coconut oil to ensure isocaloric intake. Coconut oil has a high concentration of short- (8–10 carbons) and medium-chain saturated fatty acids (12–16 carbons). Since arthritis decreases food intake, a pair-fed group of nine rats treated with coconut oil was also included. Pair-fed rats received the same amount of food (g/100 g BW) eaten on the previous day by arthritic rats treated with coconut oil.

Fenofibrate On day 4 after adjuvant injection, 18 control and 18 arthritic rats were each divided into two groups. The first group received fenofibrate (300 mg/kg BW, suspended in 500 μ l 1% carboxymethylcellulose (CMC), Sigma–Aldrich) daily by oral gavage. The second group was gavaged with vehicle (500 μ l 1% CMC). A pair-fed group of nine rats treated with vehicle was also included.

All rats were killed on day 15 after adjuvant injection and after 12 days of treatment. Trunk blood was collected in cooled tubes, allowed to clot, centrifuged and the serum was stored at –20 °C until IGF1 and IGFBP3 assays were performed. Immediately after decapitation, left gastrocnemius and liver were dissected, frozen in liquid nitrogen and stored at –80 °C until RNA extraction. Isolation and manipulation of tissues were always performed under sterile conditions.

RNA extraction and real-time PCR

Gastrocnemius or liver (100 mg) was homogenized, and total RNA was extracted using Ultraspec (Biotecx Laboratories, Inc., Houston, TX, USA), following the manufacturer's protocol. The final concentration of RNA was determined (260 nm) with a BioPhotometer (Eppendorf, Germany), and the integrity of the RNA was confirmed by agarose gel electrophoresis. First-strand cDNA synthesis was performed by 1 μ g of total RNA with a Quantiscript Reverse Transcription kit (Qiagen).

Real-time PCR for quantification of mRNA was performed on a SmartCycler (Cepheid, Sunnyvale, CA, USA) using a SYBR Green protocol on the fluorescence temperature cycler. Each real-time PCR consisted of 10 ng total RNA equivalents, 1 $\times$ Takara SYBR Green Premix Ex Taq (Takara BIO, Inc., Otsu, Shiga, Japan), and 300 nM forward and reverse primers in a reaction volume of 25.5 μ l. Primers for real-time PCR (Table 1), were obtained from Roche. 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

Table 1 Primers for real-time PCR

Gene	Primers (5'–3')	Product (bp)
18S	F: GGTGCATGGCCGTCTTA R: TCGTTCGTATCGGAATTAACC	60
Igf1	F: GCTATGGCTCCAGCATTCG R: TCCGGAAGCAACACTCATCC	62
Igfbp3	F: GGAAAGACGACGTGCATTG R: GCGTATTTGAGCTCCACGTT	78
Igfbp5	F: GGCGAGCAAACCAAGATAGA R: GGTCTCTCAGCCATCTCG	75

F, forward; R, reverse.

expressed relative to the control animals treated with coconut oil or vehicle, where the relative mRNA abundance has been arbitrarily set to 1, using cycle threshold 2($\Delta\Delta C_T$) method, with 18S as reference gene. PCR products were separated using agarose gel electrophoresis to confirm the product presence and size.

IGF1 and corticosterone determinations

Serum IGF1 was measured using the antiserum to human IGF1 (UB2–495) from Dr Underwood and Dr Van Wik, and is distributed by the NIDDK Hormone Distribution Program through the National Hormone and Pituitary Program. Levels of IGF1 were expressed in terms of rat IGF1 from Gropep Ltd (Adelaide, SA, Australia). The intra-assay coefficient of variation was 8%. All samples from the same experiment were run in the same assay. Serum corticosterone was analyzed by a commercial kit from MP Biomedicals, LLC (New York, NY, USA).

Ligand blot

Serum concentrations of IGFBP3 were measured by western blot. Serum (2 μ l) was diluted in sample buffer and boiled for 2 min at 90 °C, loaded onto 1% SDS–12.5% polyacrylamide gels, and electrophoresed under non-reducing conditions. Proteins were transferred onto nitrocellulose sheets (Hybond-C extra, Amersham, UK). The membranes were dried and blocked for 1 h with 5% non-fat dry milk, 0.1% Tween (Sigma), in Tris-buffered saline. Membranes were probed overnight at 4 °C with ^{125} I-labeled IGF1 (1.5 $\times$ 10⁶ c.p.m./ml). The nitrocellulose sheets were then washed, dried, and exposed at –80 °C to X-ray film (Kodak X-Omat AR, Eastman Kodak) and to two intensifying screens for 1–4 days according to the signal obtained. The signals of the film were quantified by densitometry using a PC Image VGA24 program for Windows. The density of the IGFBP3 band in each lane was expressed as the percentage of the mean density of sera from their respective control rats.

Statistical analysis

Statistics were computed using the statistics program STATGRAPHICS plus for Windows. Data are presented as means $\pm$ S.E.M. and were tested with ANOVA; *post hoc* comparisons were made by the LSD multiple range test. Statistical significance was set at $P < 0.05$.

Results

Effect of EPA administration

Arthritis decreased body weight gain to values lower than in pair-fed rats (Table 2). EPA administration was unable to modify body weight gain in arthritic or control rats. However, EPA treatment decreased the external signs of arthritis (Table 2). Figure 1 shows the effect of arthritis and EPA administration on serum concentration of IGF1, IGFBP3, and corticosterone. In rats treated with coconut oil, arthritis decreased serum concentrations of IGF1, whereas it increased corticosterone levels. In addition, pair-fed rats had IGF1 serum concentrations similar to arthritic rats, and corticosterone concentrations slightly lower but not significantly different. EPA administration did not modify serum concentrations of these hormones in control rats, but prevented the inhibitory effect of arthritis on serum concentrations of IGF1. Arthritis did not modify serum concentrations of IGFBP3 or its gene expression in the liver (Figs 1B and 2B). *Igf1* mRNA in the liver was also decreased in pair-fed and in arthritic rats treated with coconut oil (Fig. 2A). EPA administration did not modify *Igf1* expression in the liver of control rats, but increased *Igf1* mRNA levels in the liver of arthritic rats to levels similar to control rats (Fig. 2A).

Table 2 Effect of eicosapentaenoic acid (EPA) or fenofibrate administration, from day 4 to day 15 after adjuvant injection, on body weight gain and arthritis score in control and arthritic (AA) rats. Data are mean $\pm$ S.E.M. for 8–9 rats

	Body weight gain days 4–15 g	Arthritis score
Control – coconut oil	62 $\pm$ 3.4	
AA-coconut oil	18 $\pm$ 3.8 ^a	10.2 $\pm$ 1.1
Pair-fed	31 $\pm$ 1.7 ^a	
Control-EPA	62 $\pm$ 4.0	
AA-EPA	19 $\pm$ 5.6 ^{b,c}	6.2 $\pm$ 4.0 ^d
Control – vehicle	67 $\pm$ 2.5	
AA-vehicle	19 $\pm$ 3.6 ^a	11.1 $\pm$ 1.2
Pair-fed	38 $\pm$ 1.5 ^a	
Control-fenofibrate	70 $\pm$ 2.7	
AA-fenofibrate	32 $\pm$ 7.0 ^{b,d}	6.4 $\pm$ 1.2 ^d

^aDifferent from control rats treated with coconut oil or vehicle.

^bDifferent from control rats treated with EPA or fenofibrate.

^cDifferent from pair-fed rats.

^dDifferent from arthritic rats treated with coconut oil or vehicle.

Figure 1 Serum concentrations of IGF1 (A), IGFBP3 (B), and corticosterone (C) in control, arthritic (AA) or pair-fed (PF) rats treated with 1 g/kg EPA or 1 g/kg coconut oil. Arthritis decreased serum concentrations of IGF1 in rats treated with coconut oil ($P < 0.01$) but not in those that received EPA. Corticosterone levels were increased in the two groups of arthritic rats, treated with coconut oil or EPA ($P < 0.05$). Pair-fed rats had similar serum concentrations of IGF1 and corticosterone than arthritic rats treated with coconut oil. Data are expressed as mean $\pm$ S.E.M. for $n = 8-9$ rats per group, ^adifferent from control rats treated with coconut oil, ^bdifferent from arthritic rats treated with coconut oil, ^cdifferent from control rats treated with EPA, and ^ddifferent from pair-fed rats.

Neither arthritis nor EPA administration significantly modified *Igf1* expression in the gastrocnemius, although arthritis tended to increase it (Fig. 3A). However, arthritis induced a significant increase in *Igfbp3* gene expression in the gastrocnemius muscle in rats treated with coconut oil or EPA (Fig. 3B). Arthritis also tended to increase *Igfbp5* in the gastrocnemius of rats treated with coconut oil, but this increase was not significant. EPA administration potentiated this effect, since the arthritic rats treated with EPA had higher *Igfbp5* mRNA levels than control rats (Fig. 3C).

Effect of fenofibrate administration

In arthritic rats treated with fenofibrate body weight gain was higher and arthritis scores were lower than in arthritic rats treated with vehicle (Table 2). In control rats body weight gain was similar in the rats treated with either fenofibrate or vehicle. Fenofibrate administration to control rats decreased serum concentrations of IGF1 to similar levels found in arthritic or pair-fed rats (Fig. 4A). As expected, arthritis increased serum concentrations of corticosterone in the rats treated with vehicle. In addition, serum corticosterone levels in the control rats treated with fenofibrate were similar to those of the arthritic or pair-fed rats (Fig. 4C). Liver *Igf1* mRNA showed responses that were similar to serum concentration of IGF1, since it was decreased by arthritis

and fenofibrate treatment (Fig. 5A). Neither arthritis nor fenofibrate administration modified serum concentration of IGFBP3 or its gene expression in the liver (Figs 4B and 5B).

The effect of fenofibrate treatment on the IGF system is shown in Fig. 6. Arthritis or pair-feeding the rats did not modify *Igf1* mRNA in the gastrocnemius. Fenofibrate treatment decreased *Igf1* mRNA levels in the gastrocnemius, but this decrease was only statistically significant in the arthritic rats treated with fenofibrate (Fig. 6A). Arthritis increased both *Igfbp3* and *Igfbp5* mRNA in the gastrocnemius (Fig. 6B and C). Fenofibrate treatment prevented the stimulatory effect of arthritis on *Igfbp3* and *Igfbp5* expression in the gastrocnemius.

Discussion

Our data suggest that EPA administration can prevent the inhibitory effect of chronic inflammation on serum IGF1 and liver *Igf1*. In contrast, treatment with the PPAR α agonist, fenofibrate, did not only ameliorate arthritis-induced inhibition of IGF1, but it also decreased serum IGF1 as well as *Igf1* expression in control rats.

The decrease in liver *Igf1* mRNA and serum IGF1 in pair-fed rats on day 15 after adjuvant injection suggests that the inhibitory effect of arthritis on IGF1 is secondary to the decrease in food intake. However, on day 22 after adjuvant injection, food intake in arthritic rats is similar to that of control rats, but serum IGF1 and liver *Igf1* mRNA are still lower in arthritic rats than in control or in pair-fed rats (Castillero *et al.* 2009a). These data indicate that on day 15, the inhibitory effect of arthritis on IGF1 is due to two factors; inflammation and the lower food intake. EPA administration prevented the inhibitory effect of arthritis on IGF1 and on its expression in the liver, without increasing body weight gain. The absence of effect of EPA on body weight gain can be due to the fact that EPA does not modify food intake in arthritic rats (Volker *et al.* 2000, Castillero *et al.* 2009b). A lack of

Figure 2 Effect of 1 g/kg EPA or 1 g/kg coconut oil administration on liver *Igf1* (A) and *Igfbp3* (B) mRNA in control, arthritic (AA) and pair-fed (PF) rats. Arthritis decreased *Igf1* mRNA in rats treated with coconut oil ($P < 0.05$) but not in those treated with EPA. EPA administration increased ($P < 0.05$) *Igfbp3* mRNA in control rats, but not in arthritic rats. Data are expressed as mean $\pm$ S.E.M. for $n = 8-9$ rats per group, ^adifferent from control rats treated with coconut oil, ^bdifferent from arthritic rats treated with coconut oil, and ^ddifferent from pair-fed rats.

Figure 3 Effect of 1 g/kg EPA or 1 g/kg coconut oil administration on gastrocnemius *Igf1* (A), *Igfbp3* (B), and *Igfbp5* (C) mRNA in control, arthritic (AA) and pair-fed (PF) rats. Arthritis increased *Igfbp3* mRNA. Pair-fed rats had lower *Igf1*, *Igfbp3*, and *Igfbp5* mRNA than arthritic rats $P < 0.01$. Arthritis increased *Igfbp5* mRNA in rats treated with EPA $P < 0.01$. Data are expressed as mean $\pm$ S.E.M. for $n = 8-9$ rats per group, ^adifferent from control rats treated with coconut oil, ^bdifferent from arthritic rats treated with coconut oil, ^cdifferent from control rats treated with EPA, and ^ddifferent from pair-fed rats.

effect of EPA on body weight together with a protective effect on muscle has also been reported in dystrophic muscle degeneration (Machado *et al.* 2011). All these data suggest that the protective effect of EPA on IGF1 is not related to modifications in food intake. Furthermore, fenofibrate, which increased food intake in arthritic rats (Castillero *et al.* 2011) and body weight gain, was unable to prevent the inhibitory effect of arthritis on serum IGF1 and liver *Igf1* mRNA.

The stimulatory effect of EPA on liver *Igf1* in arthritic rats can be secondary to its anti-inflammatory effect. EPA administration decreases the external signs of arthritis and prevents arthritis-induced increase in liver TNF α (Castillero *et al.* 2009a). However, fenofibrate treatment has anti-inflammatory effects in arthritic rats similar to those of EPA (Castillero *et al.* 2011), but in the present data it was unable to restore serum IGF1 and liver *Igf1* mRNA. Differences can be due to the different effect of the compounds, EPA and fenofibrate, on *Igf1* expression, since fenofibrate decreased liver *Igf1* expression in control rats and muscle *Igf1* expression in arthritic rats.

An increase in serum concentrations of IGF1 has been reported after chronic EPA administration (Childs *et al.* 2008, Bonnet & Ferrari 2010). However, in our data EPA did not modify *Igf1* expression in the liver or serum IGF1 in control rats. These differences can be due to the fact that the stimulatory effect of EPA on serum concentrations of IGF1

has been reported after treatments longer than that in this study, 14 months in mice (Bonnet & Ferrari 2010) and 45 days in cattle (Childs *et al.* 2008). Therefore, arthritic rats with lower serum IGF1 and liver *Igf1* levels seem to be more sensitive to the stimulatory effect of EPA than control rats.

The positive effect of EPA on liver *Igf1* gene expression can also be secondary to its effect on liver cells. In this sense, EPA is able to prevent LPS-induced increase in TNF expression in both liver cells alone or in liver cells cocultured with macrophages (Hao *et al.* 2010), and LPS directly inhibits *Igf1* expression in hepatocyte cultures and in cocultures with Kupffer cells (Priego *et al.* 2006, Granado *et al.* 2008). Furthermore, EPA induces cell proliferation in primary hepatocyte cultures (Liu *et al.* 2011), and IGF1 has been reported to be an important factor in proliferation and in the response to damage in liver cell (Gatto *et al.* 2008).

Several studies in humans have shown that long-term treatment with fish oil increases lean body mass whereas it decreases fat mass (Su & Jones 1993, Noreen *et al.* 2010). The aforementioned authors reported that EPA decreases free cortisol levels, and that the decrease in cortisol can be the cause of a decrease in muscle proteolysis (Noreen *et al.* 2010). In our data, EPA administration was not able to prevent the stimulatory effect of arthritis on serum concentrations of corticosterone. The lack of effect of EPA on arthritis-induced corticosterone secretion can be due to the treatment length or to a difference in species.

Control rats treated with fenofibrate had decreased liver *Igf1* and serum IGF1 and serum corticosterone levels were similar to those of arthritic rats. The stimulatory effect of fenofibrate

Figure 4 Serum concentrations of IGF1 (A), IGFBP3 (B), and corticosterone (C) in control, arthritic (AA) or pair-fed (PF) rats treated with fenofibrate (300 mg/kg) or vehicle. Arthritis and fenofibrate decreased serum concentrations of IGF1 ($P < 0.01$). Corticosterone levels were increased in arthritic rats ($P < 0.05$). Pair-fed rats had similar serum concentrations of IGF1 and corticosterone than arthritic rats. Data are expressed as mean $\pm$ S.E.M. for $n = 8-9$ rats per group, ^adifferent from control rats treated with vehicle.

on serum corticosterone has been previously reported (Chen *et al.* 2008). Taking into account the inhibitory effect of glucocorticoids on liver *Igf1* and on serum IGF1 (Luo & Murphy 1989), the increased corticosterone secretion can be one of the mechanisms through which fenofibrate inhibits IGF1 and *Igf1* expression in control rats.

To our knowledge, there is no data about the effect of fenofibrate on liver *Igf1* and serum IGF1, whereas data on *Igf1* expression in organs other than the liver are contradictory. Activation of PPAR α by fenofibrate has an anti-cancer effect and attenuates signaling responses of IGF1R in medulloblastoma and glioma cell lines (Urbanska *et al.* 2008, Drukala *et al.* 2010). In contrast, PPAR α activation in the heart, through Wy-14643 administration, results in upregulation of *Igf1* expression in myocytes and subsequent protection against ischemia/reperfusion-induced apoptosis (El Azzouzi *et al.* 2011). A possible explanation is that either fenofibrate or Wy-14643 acts on the IGF1 system in a PPAR α -independent manner.

Similarly to fenofibrate, thiazolidinediones such as rosiglitazone and pioglitazone are PPAR γ ligands that have an antiproliferative effect and block IGF1 responses in cancer cells (Freudlsperger *et al.* 2006, He *et al.* 2006). Furthermore, rosiglitazone administration reduces serum IGF1 without modifying IGFBP3 levels in humans or mice, and also reduces *Igf1* expression in liver cells, both *in vivo* and *in vitro* (Lecka-Czernik *et al.* 2007).

The inhibitory effect of fenofibrate on liver *Igf1* and serum IGF1 observed in the present data contrasts with its protective action against muscle wasting that we found in arthritic rats (Castillero *et al.* 2011). A possible explanation can be the effects of fenofibrate on the *Igfbp3* in the gastrocnemius. As previously reported (Castillero *et al.* 2009a), arthritis increased *Igfbp3* in the gastrocnemius, but not in the liver. These data suggest that *Igf1* and *Igfbp3* regulation in the skeletal muscle is different to its regulation in the liver. The increased expression of *Igfbp3* in the gastrocnemius of arthritic rats has been related to muscle atrophy. This hypothesis is based on the fact that IGFBP3 prevents IGF1 from binding to its receptor and it is able to inhibit cell proliferation by an

Figure 5 Effect of fenofibrate (300 mg/kg) administration on liver *Igf1* (A) and *Igfbp3* (B) mRNA in control, arthritic (AA) and pair-fed (PF) rats. Arthritis and fenofibrate decreased *Igf1* mRNA ($P < 0.01$). Data are expressed as mean $\pm$ S.E.M. for $n = 8-9$ rats per group, ^adifferent from control rats treated with coconut oil and ^ddifferent from pair-fed rats.

Figure 6 Effect of fenofibrate (300 mg/kg) administration on gastrocnemius *Igf1* (A), *Igfbp3* (B), and *Igfbp5* (C) mRNA in control, arthritic (AA) and pair-fed (PF) rats. Arthritis increased *Igfbp3* and *Igfbp5* mRNA ($P < 0.01$). In arthritic rats fenofibrate administration decreased *Igf1*, *Igfbp3*, and *Igfbp5* ($P < 0.01$). Pair-fed rats had lower *Igf1*, *Igfbp3*, and *Igfbp5* mRNA than arthritic rats treated with vehicle. Data are expressed as mean $\pm$ S.E.M. for $n = 8-9$ rats per group, ^adifferent from control rats treated with coconut oil and ^bdifferent from arthritic rats treated with coconut oil.

IGF1-independent mechanism (Pampusch *et al.* 2003). IGFBP5 can also inhibit IGF1 action by preventing its binding to its receptors in the skeletal muscle (Mukherjee *et al.* 2008). Therefore, the inhibitory action of fenofibrate on *Igfbp3* and *Igfbp5* expression in the gastrocnemius muscle can explain the observed effect of fenofibrate attenuating muscle wasting in arthritic rats (Castillero *et al.* 2011).

In summary, our data indicate that EPA and fenofibrate have different effects on the IGF-IGFBPs in arthritic rats, which suggests that EPA actions on this pathway are not mediated by its PPAR α agonism. EPA administration prevented the inhibitory effect of serum IGF1 and on *Igf1* gene in the liver, whereas fenofibrate administration prevented the stimulatory effect of arthritis on gastrocnemius *Igfbp3* and *Igfbp5* expression. The effects of both compounds on the IGF-IGFBPs system can explain their previously reported protective effects against muscle wasting, although these actions are exerted through different mechanisms.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by grants from FIS no PS09/00753 and Fundación Mutua Madrileña, fellowships from Gobierno Vasco to E C (BF106.31) and from Ministerio de Educación y Ciencia to M L-M (BES-2007-16001).

Acknowledgements

The authors are indebted to Dr Matti Tolonen for the supply of EPA, to Antonio Carmona for technical assistance and to Christina Bickart for the English correction of the manuscript.

References

- Bonnet N & Ferrari SL 2010 Effects of long-term supplementation with omega-3 fatty acids on longitudinal changes in bone mass and micro-structure in mice. *Journal of Nutritional Biochemistry* **22** 665–672. (doi:10.1016/j.jnutbio.2010.05.006)
- Castillero E, Martín AI, López-Menduiña M, Granado M, Villanúa MA & López-Calderón A 2009a IGF-I system, atrogens and myogenic regulatory factors in arthritis induced muscle wasting. *Molecular and Cellular Endocrinology* **309** 8–16. (doi:10.1016/j.mce.2009.05.017)
- Castillero E, Martín AI, López-Menduiña M, Villanúa MA & López-Calderón A 2009b Eicosapentaenoic acid attenuates arthritis-induced muscle wasting acting on atrogen-1 and on myogenic regulatory factors. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **297** R1322–R1331. (doi:10.1152/ajpregu.00388.2009)
- Castillero E, Nieto-Bona MP, Fernández-Galaz C, Martín AI, López-Menduiña M, Granado M, Villanúa MA & López-Calderón A 2011 Fenofibrate, a PPAR alpha agonist, decreases atrogens and myostatin expression and improves arthritis-induced skeletal muscle atrophy. *American Journal of Physiology. Endocrinology and Metabolism* **300** E790–E799. (doi:10.1152/ajpendo.00590.2010)
- Chen X, Li M, Sun W, Bi Y, Cai M, Liang H, Yu Q, He X & Weng J 2008 Peroxisome proliferator-activated receptor alpha agonist-induced down-regulation of hepatic glucocorticoid receptor expression in SD rats. *Biochemical and Biophysical Research Communications* **368** 865–870. (doi:10.1016/j.bbrc.2008.01.152)
- Childs S, Hennessy AA, Sreenan JM, Wathes DC, Cheng Z, Stanton C, Diskin MG & Kenny DA 2008 Effect of level of dietary n-3 polyunsaturated fatty acid supplementation on systemic and tissue fatty acid concentrations and on selected reproductive variables in cattle. *Theriogenology* **70** 595–611. (doi:10.1016/j.theriogenology.2008.04.002)
- Drukala J, Urbanska K, Wilk A, Grabacka M, Wybieralska E, Del Valle L, Madeja Z & Reiss K 2010 ROS accumulation IGF-IR inhibition contribute to fenofibrate/PPARalpha-mediated inhibition of glioma cell motility *in vitro*. *Molecular Cancer* **9** 159. (doi:10.1158/1541-7786.MCR-10-0019)
- El Azzouzi H, Leptidis S, Bourajaj M, Armand AS, van der Nagel R, van Bilsen M, Da Costa Martins PA & De Windt LJ 2011 Peroxisome proliferator-activated receptor (PPAR) gene profiling uncovers insulin-like growth factor-1 as a PPAR {alpha} target gene in cardioprotection. *Journal of Biological Chemistry* **286** 14598–14607. (doi:10.1074/jbc.M111.220525)
- Evans WJ, Morley JE, Argilés J, Bales C, Baracos V, Guttridge D, Jatoi A, Kalantar-Zadeh K, Lochs H, Mantovani G *et al.* 2008 Cachexia: a new definition. *Clinical Nutrition* **27** 793–799. (doi:10.1016/j.clnu.2008.06.013)
- Forman BM, Chen J & Evans RM 1997 Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *PNAS* **94** 4312–4317. (doi:10.1073/pnas.94.9.4312)
- Freudlsperger C, Moll I, Schumacher U & Thies A 2006 Anti-proliferative effect of peroxisome proliferator-activated receptor gamma agonists on human malignant melanoma cells *in vitro*. *Anticancer Drugs* **17** 325–332. (doi:10.1097/00001813-200603000-00011)
- Gatto M, Drudi-Metalli V, Torrice A, Alpini G, Cantafora A, Blotta I & Alvaro D 2008 Insulin-like growth factor-1 isoforms in rat hepatocytes and cholangiocytes and their involvement in protection against cholestatic injury. *Laboratory Investigation* **88** 986–994. (doi:10.1038/labinvest.2008.63)
- Granado M, Martín AI, López-Menduiña M, López-Calderón A & Villanúa MA 2008 GH-releasing peptide-2 administration prevents liver inflammatory response in endotoxemia. *American Journal of Physiology. Endocrinology and Metabolism* **294** E131–E141. (doi:10.1152/ajpendo.00308.2007)
- Hao W, Wong OY, Liu X, Lee P, Chen Y & Wong KK 2010 ω-3 fatty acids suppress inflammatory cytokine production by macrophages and hepatocytes. *Journal of Pediatric Surgery* **45** 2412–2418. (doi:10.1016/j.jpedsurg.2010.08.044)
- He G, Sung YM, Digiovanni J & Fischer SM 2006 Thiazolidinediones inhibit insulin-like growth factor-I-induced activation of p70S6 kinase and suppress insulin-like growth factor-I tumor-promoting activity. *Cancer Research* **66** 1873–1878. (doi:10.1158/0008-5472.CAN-05-3111)
- Hurst S, Rees SG, Randerson PF, Catteron B & Harwood JL 2009 Contrasting effects of n-3 and n-6 fatty acids on cyclooxygenase-2 in model systems for arthritis. *Lipids* **44** 889–896. (doi:10.1007/s11745-009-3347-x)
- Ibañez De Cáceres I, Villanúa MA, Soto L, Martín AI & López-Calderón A 2000 IGF-I and IGF-I-binding proteins in rats with adjuvant-induced arthritis given recombinant human growth hormone. *Journal of Endocrinology* **165** 537–544. (doi:10.1677/joe.0.1650537)
- James MJ & Cleland LG 1997 Dietary n-3 fatty acids and therapy for rheumatoid arthritis. *Seminars in Arthritis and Rheumatism* **27** 85–97. (doi:10.1016/S0049-0172(97)80009-1)
- Lecka-Czernik B, Ackert-Bicknell C, Adamo ML, Marmolejos V, Churchill GA, Shockley KR, Reid IR, Grey A & Rosen CJ 2007 Activation of peroxisome proliferator-activated receptor gamma (PPARgamma) by rosiglitazone suppresses components of the insulin-like growth factor regulatory system *in vitro* and *in vivo*. *Endocrinology* **148** 903–911. (doi:10.1210/en.2006-1121)
- Leslie CA, Gonnerman WA, Ullman MD, Hayes KC, Franzblau C & Cathcart ES 1985 Dietary fish oil modulates macrophage fatty acids and decreases arthritis susceptibility in mice. *Journal of Experimental Medicine* **162** 1336–1349. (doi:10.1084/jem.162.4.1336)
- Liu WM, Shi FX, Lu LZ, Zhang C, Liu YL, Zhang J, Tao ZR, Shen JD, Li GQ, Wang DQ *et al.* 2011 Effects of linoleic acid and eicosapentaenoic acid on cell proliferation and lipid-metabolism gene expression in primary duck hepatocytes. *Molecular and Cellular Biochemistry* **352** 19–24. (doi:10.1007/s11010-011-0735-3)
- López-Calderón A, Soto L & Martín AI 1999 Chronic inflammation inhibits GH secretion and alters the serum insulin-like growth factor system in rats. *Life Sciences* **65** 2049–2060. (doi:10.1016/S0024-3205(99)00472-5)
- López-Menduiña M, Martín AI, Castillero E, Villanúa MA & López-Calderón A 2010 Systemic IGF-I administration attenuates the inhibitory effect of chronic arthritis on gastrocnemius mass and decreases atrogen-1 and IGFBP-3. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **299** R541–R551. (doi:10.1152/ajpregu.00211.2010)
- Luo J & Murphy LJ 1989 Dexamethasone inhibits growth hormone induction of insulin-like growth factor-I (IGF-I) messenger ribonucleic acid (mRNA) in hypophysectomized rats and reduces IGF-I abundance in the intact rat. *Endocrinology* **125** 165–171. (doi:10.1210/endo-125-1-165)
- Machado RV, Mauricio AF, Taniguti AP, Ferretti R, Neto HS & Marques MJ 2011 Eicosapentaenoic acid decreases TNF-α and protects dystrophic muscles of mdx mice from degeneration. *Journal of Neuroimmunology* **232** 145–150. (doi:10.1016/j.jneuroim.2010.10.032)
- Michalik L & Wahli W 2008 PPARs mediate lipid signaling in inflammation and cancer. *PPAR Research* **2008** Article ID 134059. (doi:10.1155/2008/134059)
- Morley JE, Thomas DR & Wilson MM 2006 Cachexia: pathophysiology and clinical relevance. *American Journal of Clinical Nutrition* **83** 735–743.
- Mukherjee A, Wilson EM & Rotwein P 2008 Insulin-like growth factor (IGF) binding protein-5 blocks skeletal muscle differentiation by inhibiting IGF actions. *Molecular Endocrinology* **22** 206–215. (doi:10.1210/me.2007-0336)
- Noreen EE, Sass MJ, Crowe ML, Pabon VA, Brandauer J & Averill LK 2010 Effects of supplemental fish oil on resting metabolic rate, body composition, and salivary cortisol in healthy adults. *Journal of the International Society of Sports Nutrition* **7** 31. (doi:10.1186/1550-2783-7-31)
- Okamoto H, Iwamoto T, Kotake S, Momohara S, Yamanaka H & Kamatani N 2005 Inhibition of NF-kappaB signaling by fenofibrate, a peroxisome proliferator-activated receptor-alpha ligand, presents a therapeutic strategy for rheumatoid arthritis. *Clinical and Experimental Rheumatology* **23** 323–330.

- Pampusch MS, Kamanga-Sollo E, White ME, Hathaway MR & Dayton WR 2003 Effect of recombinant porcine IGF-binding protein-3 on proliferation of embryonic porcine myogenic cell cultures in the presence and absence of IGF-I. *Journal of Endocrinology* **176** 227–235. (doi:10.1677/joe.0.1760227)
- Pawer A & Jump DB 2003 Unsaturated fatty acid regulation of peroxisome proliferator-activated receptor α activity in rat primary hepatocytes. *Journal of Biological Chemistry* **278** 35931–35939. (doi:10.1074/jbc.M306238200)
- Priego T, Granado M, Castillero E, Martín AI, Villanúa MA & López-Calderón A 2006 Nitric oxide production by hepatocytes contributes to the inhibitory effect of endotoxin on insulin-like growth factor I gene expression. *Journal of Endocrinology* **190** 847–856. (doi:10.1677/joe.1.06938)
- Simopoulos AP 2002 Omega-3 fatty acids in inflammation and autoimmune diseases. *Journal of the American College of Nutrition* **21** 495–505.
- Soto L, Martín AI, Millán S, Vara E & López-Calderón A 1998 Effects of endotoxin lipopolysaccharide administration on the somatotrophic axis. *Journal of Endocrinology* **159** 239–246. (doi:10.1677/joe.0.1590239)
- Su W & Jones PJ 1993 Dietary fatty acid composition influences energy accretion in rats. *Journal of Nutrition* **123** 2109–2114.
- Tanaka H, Ueta Y, Yamashita U, Kannan H & Yamashita H 1996 Biphasic changes in behavioral, endocrine, and sympathetic systems in adjuvant arthritis in Lewis rats. *Brain Research Bulletin* **39** 33–37. (doi:10.1016/0361-9230(95)02037-3)
- Tashiro T, Yamamori H, Takagi K, Hayashi N, Furukawa K & Nakajima N 1998 n-3 versus n-6 polyunsaturated fatty acids in critical illness. *Nutrition* **14** 551–553. (doi:10.1016/S0899-9007(98)00048-3)
- Touati G, Prieur AM, Ruiz JC, Noel M & Czernichow P 1998 Beneficial effects of one-year growth hormone administration to children with juvenile chronic arthritis on chronic steroid therapy. I. Effects on growth velocity and body composition. *Journal of Clinical Endocrinology and Metabolism* **83** 403–409. (doi:10.1210/jc.83.2.403)
- Urbanska K, Pannizzo P, Grabacka M, Croul S, Del Valle L, Khalili K & Reiss K 2008 Activation of PPAR α inhibits IGF-I-mediated growth and survival responses in medulloblastoma cell lines. *International Journal of Cancer* **123** 1015–1024. (doi:10.1002/ijc.23588)
- Volker DH, FitzGerald PE & Garg ML 2000 The eicosapentaenoic to docosahexaenoic acid ratio of diets affects the pathogenesis of arthritis in Lew/SSN rats. *Journal of Nutrition* **130** 559–565.
- Zhao Y, Joshi-Barve S, Barve S & Chen LH 2004 Eicosapentaenoic acid prevents LPS-induced TNF- α expression by preventing NF- κ B activation. *Journal of the American College of Nutrition* **23** 71–78.

Received in final form 13 June 2011

Accepted 29 June 2011

Made available online as an Accepted Preprint
29 June 2011