Tumour necrosis factor blockade did not prevent the increase of muscular muscle RING finger-1 and muscle atrophy F-box in arthritic rats

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Abstract

Chronic inflammation is associated with a decrease in body weight and cachexia, which is characterized by anorexia and skeletal muscle wasting. The expression of atrogens muscle RING finger-1 (MuRF-1) and muscle atrophy F-box (MAFbx) are increased in muscle atrophy and it is known that tumour necrosis factor (TNF) regulates skeletal muscle loss through TNF receptor p55 (TNFR1). The aim of this study was to examine the effect of polyethylene glycol linked to soluble TNFR1 (PEG-sTNFR1) on gene expression of the atrogens MuRF-1 and MAFbx in skeletal muscle of arthritic rats. Rats were injected with Freund's adjuvant and, 15 days later, arthritic and control rats were injected daily with PEG-sTNFR1 (1 mg/kg, s.c.) or saline for 8 days. Arthritis

decreased body weight gain, the weight of skeletal muscle and adipose mass. PEG-sTNFR1 administration increased body weight gain and adipose mass of arthritic rats; however, it did not modify the skeletal muscle weight. The gene expression of TNF- α , MuRF1 and MAFbx, IGF-I and IGFBP-5 were increased in the skeletal muscle of arthritic rats, and the administration of PEG-sTNFR1 did not modify these parameters. These data suggest that the anti-TNF agent PEG-sTNFR1 did not prevent the increase in E3 ubiquitin-ligating enzymes, MuRF1 and MAFbx, gene expression in the skeletal muscle of arthritic rats.

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Introduction

Adjuvant-induced arthritis is an animal model for chronic inflammation and rheumatoid arthritis characterized by a decrease in body weight and cachexia (Roubenoff *et al.* 1997, Ibáñez de Cáceres *et al.* 2000). Cachexia, which also prevails in other situations such as burns, sepsis and cancer, is characterized by anorexia and tissue wasting, reflected essentially in a loss of adipose tissue and skeletal muscle.

The skeletal muscle loss can be due to both the reduction in protein synthesis and the increase in myofibrillar protein breakdown. The increased protein breakdown seems to play a major role in muscle wasting as observed in rats with sepsis (Hasselgren *et al.* 1989) and in cancer patients (Tisdale 2000). Skeletal muscle contains three major proteolytic systems; one of them is the ubiquitin–proteasome-dependent pathway that mediates the degradation of the most abundant contractile proteins (Mitch & Goldberg 1996). Ubiquitin targets specific protein substrates for degradation and this action involves the ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzymes (E2), and, in general, ubiquitin-protein ligases (E3) (Hershko *et al.* 2000). There are two new ubiquitin ligases that seem to be specifically involved in myofibrillar protein degradation, the mouse atrophy gene-1 (atrogin-1; Gomes

et al. 2001), also described as the rat muscle atrophy F-box (MAFbx; Bodine *et al.* 2001), and the rat muscle RING finger-1 (MuRF-1; Bodine *et al.* 2001). Their expression is increased in catabolic conditions that result in muscle atrophy (Bodine *et al.* 2001, Gomes *et al.* 2001, Dehoux *et al.* 2003). Recently, Lecker *et al.* (2004) has confirmed that these two atrogens are clearly essential in the process of muscle wasting. In addition, recent studies from our laboratory have provided evidence that arthritis-induced muscle cachexia mainly reflects increased ubiquitin–proteasome-dependent proteolysis and is associated with up-regulated gene expression of E3 ubiquitin-ligating enzymes MuRF-1 and MAFbx in skeletal muscle (Granado *et al.* 2005).

Muscle proteolysis, in particular, activation of the ubiquitin–proteasome system, has been reported to be regulated by tumour necrosis factor (TNF- α) (Llovera *et al.* 1997) and insulin-like growth factor (IGF-I) (Chrysos & Underwood 1999). Anti-TNF antibodies prevented increased protein breakdown and increased expression of ubiquitin in skeletal muscle from rats with hepatoma (Costelli *et al.* 1993, Llovera *et al.* 1996). Moreover, inhibition of TNF production *in vivo* by xanthine derivatives, such as pentoxifylline or torbafylline prevented muscle wasting and suppressed the enhanced ubiquitin–proteasome-dependent proteolysis in

septic and cancer rats (Vary *et al.* 1999, Combaret *et al.* 2002). In arthritic rats, the increase in muscular *MuRF-1* and *MAFbx* gene expression was accompanied by the increase in the gene expression of TNF- α (Granado *et al.* 2005). Catabolic actions of TNF can be exerted in different ways. One possibility is that TNF- α acts directly on skeletal muscle to induce muscle catabolism. Alternatively, TNF- α can act indirectly, stimulating skeletal muscle catabolism by modifying hormones that regulate protein turnover, such as IGF-I (Fernández-Celemín *et al.* 2002) or by inducing anorexia (Tracey *et al.* 1990).

The role of IGF-I in muscle cachexia remains unclear, since this growth factor stimulates muscle protein synthesis, but it does not prevent the sepsis-induced increase in muscle protein breakdown (Fang *et al.* 2000). However, IGF-I has also been reported to inhibit the ubiquitin-proteasome system in skeletal muscle in a catabolic state induced by dexamethasone (Chrysis & Underwood 1999). The actions of IGF-I are modulated locally by the IGF-binding proteins (IGFBPs). IGFBP-5 binds to the extracellular matrix and modulates the muscle's response to IGF-I (Schneider *et al.* 2002). A down-regulation of IGFBP-5 has been reported by microarray studies in several models of muscular wasting (Lecker *et al.* 2004). However, in arthritic rats, we found that catabolic response in skeletal muscle was associated with increased gene expression of IGF-I and IGFBP-5 (Granado *et al.* 2005).

Since most of the TNF- α effects on skeletal muscle protein turnover are mediated through TNF receptor p55 (TNFRI) (Llovera *et al.* 1998), in the present study we tested the hypothesis that administration of high affinity anti-TNF agent, polyethylene glycol linked to soluble TNFRI (PEG-sTNFRI) inhibits the muscular atrophy and the increase in E3 ubiquitin-ligating enzymes MuRF-1 and MAFbx gene expression in skeletal muscle of arthritic rats. Since catabolic response in skeletal muscle was associated with increased gene expression of TNF- α , IGF-I and IGFBP-5 in our previous study (Granado *et al.* 2005), mRNA levels for TNF- α , IGF-I and IGFBP-5 in gastrocnemius muscle were also determined.

Materials and Methods

Animals and experimental design

Male Wistar rats were housed three or four per cage and were given a regular supply of food and water and allowed to feed *ad libitum*, under constant conditions of temperature (20–22 °C) and light (lights on from 0730 to 1930 h). The procedures followed the guidelines recommended by the EU for the care and use of laboratory animals. Control and arthritic rats were purchased from Charles River (Barcelona, Spain). Arthritis was induced by an intradermal injection of a suspension of 1 mg heat-killed *Mycobacterium butyricum* in incomplete Freund's adjuvant. The injection was given in the plantar surface of the right hind paw under ketamine (75 mg/kg) + diazepam (5 mg/kg) anaesthesia. Control animals were injected with vehicle (paraffin oil). Fifteen days after adjuvant injection,

20 control and 24 arthritic rats were divided into two groups, one injected daily with 1 mg/kg s.c. PEG-sTNFRI (Amgen Inc, Thousand Oaks, CA, USA) and the other with 250 μ l saline, until day 22 after adjuvant injection. At this dose, this TNF-binding complex is able to block the systemic TNF- α response (evaluated by its cytotoxicity activity) as well as plasma TNF- α bioactivity in baboons challenged with a lethal *Escherichia coli* bacteraemia (Solorzano *et al.* 1998). Assessment of arthritis was performed by measuring the arthritis index of each animal, which was scored by grading each paw from 0 to 4. Grading was determined as the following: 0, neither erythema nor swelling; 1, slight erythema or swelling of one or more digits; 2, swelling of 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 each limb, the maximum value being 16 (Tanaka *et al.* 1996). Rats having an arthritis index score below 8 on day 15 (1 out of 25 rats) were excluded from the experiment. All rats were weighed daily. Food intake per cage was calculated daily by measuring the difference between the initial and the remaining amount of pellets in the feeder, and expressed as grams per 100 g of body weight throughout the 8 days of treatment.

On day 22 after adjuvant or vehicle injection, all rats were killed by decapitation, 2.5 h after the last PEG-sTNFRI or saline injection. Gastrocnemius muscle from the left paw was frozen in liquid nitrogen and stored at –80 °C. Gastrocnemius muscle from the right paw and epididymal fat were removed, dissected and weighed.

RNA extraction and real-time PCR

Total RNA from skeletal muscle was extracted by the guanidine thiocyanate method using a commercial reagent (Ultraspec RNA, Biotecx Laboratories, Houston, TX, USA). The extracted RNA was dissolved in diethyl pyrocarbonate water with 0.1% SDS and quantified at 260 nm; RNA integrity was confirmed by agarose gel electrophoresis. For RT-PCR analysis, 2 μ g skeletal muscle mRNA were reverse transcribed in a total volume of 30 μ l at 37 °C for 60 h with 125 units of Moloney murine leukaemia virus RT (Maxim Bioch, San Francisco, CA, USA). Each RT-PCR reaction consisted of 2.5 μ l cDNA, SYBR Premix Ex Taq (Takara Otsu, Shiga, Japan), and 300 nM forward and reverse primers in a reaction volume of 25 μ l. Reactions were carried out on a SmartCycler (Cepheid, Sunnyvale, CA, USA). Primers for PCR were obtained from previously published sequences of TNF- α , MuRF-1 and IGF-I (Dehoux *et al.* 2003), r18S (Bar *et al.* 2003) or using the rat GenBank and the EXIQON ProbeLibrary IGFBP-5 and MAFbx. Primers were designed to span a single sequence derived from two exons (i.e. separated by an intron in genomic DNA and primary RNA transcripts to minimize amplification). Parameters included an initial activation of hotStar Taq DNA polymerase at 95 °C for 10 s, followed by 40 cycles of denaturation at 94 °C for 15 s, annealing at 52 °C (MAFbx), 53 °C (MuRF-1 and IGFBP-5), 55 °C (IGF-I), 57 °C (TNF- α), and 60 °C (r18S)

for 30 s, and extension at 72 °C for 30 s. Specific amplification was confirmed by the presence of single peak in the melting curve plots. In addition, the PCR products were analysed in agarose gel electrophoresis. Results were calculated as percentage of control rats injected with saline, using the $\Delta\Delta C_T$ method (Livak & Schmittgen 2001) with *r18S* as the control gene.

Statistical analysis

Statistics were computed using the statistics program STATGRAPHICS plus for Windows (Manugistic, Inc., Rockville, MD, USA). Data are presented as mean $\pm$ S.E.M. and were analysed by one-way ANOVA followed by least significant difference (LSD) multiple range test or two-way ANOVA with arthritis and PEG-sTNFR1 as factors. When the two-way ANOVA indicated a significant interaction between factors, individual means were compared with LSD multiple range test. Epididymal fat weight and TNF- α mRNA data were subjected to log transformation, since variances showed a non-normal distribution after Kolmogorov–Smirnov test. Statistical significance was set at $P < 0.05$.

Results

As shown in Fig. 1, arthritic rats injected with saline gained less body weight than controls rats, between days 15 and 22 after adjuvant injection ($P < 0.01$). This decrease in body weight gain was concomitant with a significant ($P < 0.05$ and 0.01) decrease in food intake (Fig. 1, upper and lower panel). PEG-sTNFR1 administration to arthritic rats induced an increase in body weight gain as compared with arthritic rats injected with saline ($P < 0.05$ on days 1–3 and $P < 0.01$ on days 4–7). The stimulatory effect of PEG-sTNFR1 treatment on body weight is associated with an increase in food intake, since from days 2 to 7 of the experiment, food intake was statistically increased in arthritic rats treated with PEG-sTNFR1 ($P < 0.05$ and 0.01) as compared with arthritic rats injected with saline. The 8-day administration of PEG-sTNFR1 did not modify body weight gain or food intake in control rats (Fig. 1).

Arthritis induced a marked decrease in the gastrocnemius weight and in the epididymal fat weight ($P < 0.01$; Table 1). PEG-sTNFR1 administration did not modify the gastrocnemius weight. However, this anti-TNF agent increased the epididymal fat ($P < 0.05$; Table 1).

There was a significant increase ($P < 0.05$) in TNF- α mRNA in the skeletal muscle of arthritic rats injected with saline. PEG-sTNFR1 administration did not modify the TNF- α mRNA in arthritic rats, but it increased TNF- α mRNA in control rats even though this increase was not statistically significant (Fig. 2).

As shown in Fig. 3, arthritis induced a significant increase in the two ubiquitin ligases MuRF-1 ($P < 0.05$) and MAFbx ($P < 0.01$) gene expression in the skeletal muscle. After PEG-sTNFR1 administration, gene expression of MuRF-1

Figure 1 Effect of PEG-sTNFR1 (1 mg/kg s.c.) or saline administration to control or arthritic rats (AA) on body weight gain and food intake during the 8 days of treatment (between days 15 and 22 after adjuvant injection). Values shown are mean $\pm$ S.E.M.; $n = 9$ –12 rats for body weight gain and $n = 3$ –4 cages for food intake. * $P < 0.05$, $^{\dagger}P < 0.01$ arthritis saline vs control saline, $^{\ddagger}P < 0.05$, $^{\S}P < 0.01$ arthritis PEG-sTNFR1 vs arthritis saline (one-way ANOVA plus LSD multiple range test).

and MAFbx in the skeletal muscle of control and arthritic rats was similar to values found after saline administration.

The effects of arthritis and PEG-sTNFR1 on IGF-I mRNA and IGFBP-5 mRNA in the skeletal muscle are shown in Fig. 4. Arthritis induced an increase in both IGF-I mRNA ($P < 0.05$) and IGFBP-5 mRNA ($P < 0.01$) in the skeletal muscle, whereas PEG-sTNFR1 administration did not modify the mRNA of IGF-I and IGFBP-5 in both control and arthritic rats.

Discussion

Our results show that treatment with PEG-sTNFR1 resulted in no change in *MuRF-1* and *MAFbx* gene expression in

Table 1 Gastrocnemius and epididymi fat weight in control and arthritic (AA) rats treated with saline or PEG-sTNFRI from days 15 to 22 after adjuvant or vehicle injection

	Control saline	Control PEG-sTNFRI	AA saline	AA PEG-sTNFRI
Gastrocnemius (mg)	1564±34	1641±42	500±26	535±39
Epididymi fat log (mg)	3.55±0.04	3.62±0.07	3.10±0.03	3.22±0.03

Two-way ANOVA revealed that there was no interaction between the effects of arthritis and PEG-sTNFRI administration on gastrocnemius weight ($F_{1,43}=0.32$, $P=0.57$) and on epididymi fat log weight ($F_{1,21}=0.37$, $P=0.54$). Arthritis decreased the gastrocnemius weight ($F_{1,43}=886$, $P<0.01$) and the epididymi fat weight ($F_{1,21}=100$, $P<0.01$). PEG-sTNFRI administration increased the epididymi fat weight ($F_{1,21}=5.04$, $P<0.05$).

gastrocnemius muscle. These effects could be the result of the lack of effect of this anti-TNF agent on muscular *TNF-α*, *IGF-I* and *IGFBP-5* gene expression. Moreover, data indicate that the effects on *MuRF-1* and *MAFbx* gene expression in gastrocnemius muscle are independent of food intake.

The weight lost by the arthritic rats is not secondary to the reduction observed in food intake (Ibáñez de Cáceres *et al.* 2000, Granado *et al.* 2006). The decrease in body weight in arthritic rats can be seen in the decrease in skeletal muscle weight and in the adipose mass. This muscular atrophy has been reported in adjuvant-arthritic rats (Hamada *et al.* 2000), septic rats (Cooney *et al.* 1999) and rats treated with TNF (Hoshino *et al.* 1991). In TNF-infused rats, the loss of gastrocnemius mass has been ascribed to food intake reduction (Hoshino *et al.* 1991). This result contrasts with that obtained in the present study, since administration of PEG-sTNFRI to arthritic rats stimulates food intake but does not alter gastrocnemius weight. These results support previous observations (Roubenoff *et al.* 1997) which demonstrate that although adjuvant arthritis decreases food intake, cachexia is not the result of the decrease in caloric intake. PEG-sTNFRI administration did not change gastrocnemius weight in control or in arthritic rats. Similarly, administration of

Figure 2 *TNF-α* mRNA in skeletal muscle of control and arthritic (AA) rats treated with PEG-sTNFRI (1 mg/kg s.c.) or saline. There was an interaction between the effects of arthritis and PEG-sTNFRI ($F_{1,30}=4.27$, $P<0.05$), as PEG-sTNFRI increased muscular *TNF-α* mRNA in control but not arthritic rats. Each bar represents the mean $\pm$ s.e.m. for 8–10 rats per group. * $P<0.05$ vs control saline (two-way ANOVA plus LSD multiple range test).

Figure 3 Effect of PEG-sTNFRI (1 mg/kg s.c.) or saline administration on skeletal muscle *MuRF-1* (upper panel) and *MAFbx* (lower panel) gene expression in control and arthritic (AA) rats. There was an interaction between the effects of arthritis and PEG-sTNFRI on muscular *MuRF-1* gene expression ($F_{1,33}=4.95$, $P<0.05$), as PEG-sTNFRI increased muscular *MuRF-1* in arthritic but not in control rats. There was no interaction between the effects of arthritis and PEG-sTNFRI on muscular *MAFbx* gene expression ($F_{1,34}=0.04$, $P=0.84$). Arthritis increased *MAFbx* ($F_{1,34}=11.56$, $P<0.01$) gene expression in the skeletal muscle. Each bar represents the mean $\pm$ s.e.m. for 8–10 rats per group. * $P<0.05$ vs control saline, † $P<0.01$ vs control PEG-sTNFRI (two-way ANOVA plus LSD multiple range test).

Figure 4 Skeletal muscle IGF-I mRNA (upper panel) and IGFBP-5 mRNA (lower panel) in control and arthritic (AA) rats injected with PEG-sTNFR1 (1 mg/kg s.c.) or saline. There was no interaction between the effects of arthritis and PEG-sTNFR1 on muscular IGF-I mRNA ($F_{1,32}=0.00$, $P=0.95$) and on muscular IGFBP-5 mRNA ($F_{1,35}=0.06$, $P=0.80$). Arthritis increased IGF-I mRNA ($F_{1,32}=7.02$, $P<0.05$) and IGFBP-5 mRNA ($F_{1,35}=17.74$, $P<0.01$) in the skeletal muscle. Each bar represents the mean $\pm$ S.E.M. for 9–11 rats per group (two-way ANOVA).

this TNF- α inhibitory protein did not modify gastrocnemius weight in control rats, although this inhibitory protein increased it in septic rats (Cooney *et al.* 1999). The increase in body weight gain in arthritic rats after PEG-sTNFR1 administration is reflected in the increase in fat mass. There is evidence that TNF- α can act through the TNF- α receptor (TNFR1) to inhibit adipogenesis (Warne 2003).

Arthritis decreased gastrocnemius weight and increased the gene expression of ubiquitin ligases MuRF-1 and MAFbx in the skeletal muscle as previously shown (Granado *et al.* 2005). Furthermore, in tumour-bearing mice, the decrease in gastrocnemius muscle mass was associated with increased

muscle levels of E3 ubiquitin-ligases (Hitt *et al.* 2005). These data suggest that the decrease in skeletal muscle weight is secondary to an enhanced protein degradation pathway, the ubiquitin–proteasome proteolytic pathway. PEG-sTNFR1 administration to arthritic rats increased food intake, but it did not prevent the activation of ubiquitin ligase gene expression. These results suggest that *MuRF-1* and *MAFbx* gene expression in the skeletal muscle are independent of food intake. PEG-sTNFR1 administration did not change muscular *MuRF-1* and *MAFbx* gene expression or gastrocnemius weight in arthritic rats. However, suppression of TNF by pentoxifylline prevented the inhibition of protein synthesis and stimulation of protein degradation as well as the decrease in epitrochlearis muscle weight during sepsis (Vary *et al.* 1999). These authors suggest that the effect of pentoxifylline in the regulation of protein turnover during sepsis does not appear to be dependent on food intake (Vary *et al.* 1999).

IGF-I controls muscle mass by enhancing myofibre hypertrophy and stimulating myogenesis (Musaro *et al.* 2001). However, it has been reported (Foulstone *et al.* 2001) that the effects of IGF-I on muscle may depend on the cytokine environment in such a way that in the presence of TNF- α , IGF-I induces apoptosis. The increase in *IGF-I* gene expression in skeletal muscle of arthritic rats does not correlate with the decrease observed in the muscle of lipopolysaccharide (LPS)-treated rats (Lang *et al.* 2000, Fernández-Celemín *et al.* 2002) and fasting rats (Dehoux *et al.* 2004). The increase in *MuRF-1* and *MAFbx* gene expression found in the skeletal muscle of arthritic rats (Granado *et al.* 2005) has also been found in the muscle of LPS-injected rats (Dehoux *et al.* 2003). Muscle mRNA content of MAFbx was also increased in other catabolic situations, such as fasting and diabetes (Dehoux *et al.* 2004). Since IGF-I has been reported to inhibit MAFbx expression in the muscles of fasting and diabetic rats (Dehoux *et al.* 2004), the increase in IGF-I in the muscle of arthritic rats should be followed by a decrease in muscular *MuRF-1* and *MAFbx* gene expression. Since this is not the case, there is a possibility that arthritic muscles have become resistant to IGF-I, as has been observed in experimental models of sepsis (Fang *et al.* 2000) and chronic renal failure (Ding *et al.* 1996). This effect can be exerted by muscular TNF- α , since this cytokine acts on muscle cells inducing a state of IGF-I receptor resistance (Broussard *et al.* 2003). Moreover, anti-TNF therapy improves glucocorticoid-induced IGF-I resistance in rheumatoid arthritis (Sarzi-Putini *et al.* 2006).

On the other hand, muscular IGFBP-5 could also contribute to the loss of the beneficial IGF-I effect, since this protein can inhibit or enhance IGF-I activity (Schneider *et al.* 2002). The widespread growth retardation demonstrated by transgenic mice that overexpress IGFBP-5 suggests that its predominant effect is inhibitory (Salih *et al.* 2004). In arthritic rats, we found an increase in muscular *IGFBP-5* gene expression, which has been previously reported in muscular atrophy (Awede *et al.* 1999) and in chondrocytes isolated from osteoarthritic cartilage (Olney *et al.* 1996). The increase in

IGFBP-5 in arthritic rats is probably not secondary to the increase in muscular IGF-I, since it has been recently observed in transgenic mice that overexpress IGF-I in muscle that IGFBP-5 mRNA expression was not affected (Musaro *et al.* 2001, Oliver *et al.* 2005). However, the increase in IGFBP-5 in arthritic rats could be secondary to the increase in TNF- α release, since TNF- α can stimulate IGFBP-5 in articular chondrocytes (Sunic *et al.* 1998). Moreover, PEG-sTNFRI administration did not modify TNF- α gene expression in gastrocnemius muscle as it did with the gene expression of IGFBP-5 in this muscle. These results suggest that the lack of effect of PEG-sTNFRI administration on muscular IGF-I gene expression could be due to the lack of effect of PEG-sTNFRI on muscular TNF- α and IGFBP-5.

In accordance with our results, the same dose of PEG-sTNFRI did not modify gastrocnemius IGF-I gene expression in rats with sepsis (Yumet *et al.* 2002) and in burned rats (Lang *et al.* 2002). This effect seems to be specific for muscular TNF, since the inhibition of TNF production by PEG-sTNFRI administration was able to attenuate the decrease in hepatic IGF-I mRNA in arthritic (Granado *et al.* 2006) and septic rats (Yumet *et al.* 2002). In addition, it was also able to attenuate the increase in hepatic IGF-I mRNA in burned rats (Lang *et al.* 2002).

The lack of effect of PEG-sTNFRI therapy on TNF- α mRNA in the skeletal muscle of arthritic rats is in agreement with the results observed on TNF- α mRNA in the synovium and in the inguinal lymph nodes after the administration of 10 mg/kg PEG-sTNFRI in adjuvant arthritis (Bush *et al.* 2002). In this study, PEG-sTNFRI therapy attenuated the illness, but it did not modify mRNA expression of synovial TNF.

Inhibition of TNF- α synthesis by xanthine derivatives prevents muscle protein breakdown in septic rats (Vary *et al.* 1999) and prevents cancer and sepsis-induced increase in muscular ubiquitin gene expression (Combaret *et al.* 2002). The discrepancy between these findings and our results might be due to the fact that xanthine derivatives inhibit TNF- α synthesis, thus decreasing local and circulating TNF; but in our study, PEG-sTNFRI administration only prevents the effect of circulating TNF. However, other authors have found that inhibiting TNF action by anti-TNF antibodies also reverted the increased muscle ubiquitin gene expression in tumour-bearing rats (Llovera *et al.* 1996). The differences in results could be explained by the fact that in tumour-bearing rats there is a substantial decrease in skeletal muscle TNF- α gene expression (Figueras *et al.* 2005), while in our study we found an increase in muscular TNF- α gene expression.

Although PEG-sTNFRI was effective as an anti-inflammatory agent, improving paw volume and arthritis index scores (Granado *et al.* 2006) as well as increasing body weight gain, it was not able to block the increase in the gene expression of TNF- α in the muscle of arthritic rats. It has been postulated that TNF- α exerts its catabolic effects in skeletal muscle in a paracrine/autocrine manner

(Fernández-Celemín *et al.* 2002). Therefore, the gene expression of E3 ubiquitin ligases MuRF-1 and MAFbx in the muscle of arthritic rats was not changed after PEG-sTNFRI administration probably as a consequence of the lack of effect of this protein complex on muscular TNF- α .

In conclusion, the administration of PEG-sTNFRI to arthritic rats does not modify the weight of gastrocnemius muscle and muscular *MuRF1* and *MAFbx* gene expression, although it increases body weight and fat mass.

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