Control of adipose tissue lipid metabolism by tumor necrosis factor-α in rainbow trout (*Oncorhynchus mykiss*)

A Albalat[1], C Liarte[2], S MacKenzie[2], L Tort[2], J V Planas[1] and I Navarro[1]

[1]Departament de Fisiologia, Facultat de Biologia, Universitat de Barcelona, Avda Diagonal 645, Barcelona 08028, Spain

[2]Departament de Biologia Cel.lular, de Fisiologia i d'Immunologia de la Universitat Autònoma de Barcelona, Barcelona, Spain

(Requests for offprints should be addressed to I Navarro; Email: mnavarro@ub.edu)

Abstract

Tumor necrosis factor-α (TNFα) is a cytokine with multiple biological functions which, in mammals, has been shown to modulate muscle and adipose tissue metabolism. In fish, TNFα has been identified in several species. However, few studies have examined the role of TNFα in fish outside the immune system. In this study, we assessed the effects of human recombinant TNFα and conditioned media from rainbow trout lipopolysaccharide (LPS)-stimulated macrophages (LPS-MCM) on lipolysis in isolated rainbow trout adipocytes. Furthermore, we studied the effects of an LPS injection *in vivo* on lipid metabolism. In our study, human recombinant TNFα stimulated lipolysis in trout adipocytes in a time- and dose-dependent manner. Similarly, LPS-MCM stimulated lipolysis in trout adipocytes when compared with control conditioned medium. Experiments using specific inhibitors of the MAP kinase pathway showed that p44/42 and p38 are partially involved in the lipolytic effects of TNFα. On the other hand, adipocytes from LPS-injected rainbow trout showed higher basal lipolysis than adipocytes from control fish after 24 h, while this effect was not seen at 72 h. Furthermore, lipoprotein lipase (LPL) activity in adipose tissue of LPS-injected fish was lower than in the controls at 24 h. These data suggest that TNFα plays an important role in the control of lipid metabolism in rainbow trout by stimulating lipolysis *in vitro* and *in vivo* and by down-regulating LPL activity of adipose tissue *in vivo*.

Journal of Endocrinology (2005) **184,** 527–534

Introduction

Tumor necrosis factor (TNF) belongs to a large family of structurally related proteins called the TNF ligand superfamily. In mammals, two forms of TNF have been identified and characterized (called α and β) which share 30% of protein sequence homology and which are known to act through the same receptors (Vilcek & Lee 1991).

In fish, the first gene identified that codified for TNFα was found in the Japanese flounder (*Paralychthys olivaceus*) (Hirono *et al.* 2000). Further studies have identified and characterized TNFα mRNA in rainbow trout (*Oncorhynchus mykiss*) (Laing *et al.* 2001), brook trout (*Salvelinus fontinalis*) (Bobe & Goetz 2001), carp (*Cyprinus carpio*) (Saeji *et al.* 2003) and sea bream (*Sparus aurata*) (Garcia-Castillo *et al.* 2002). More recently, Zou *et al.* (2002) have provided evidence for the presence of two different TNF genes in rainbow trout, the novel TNF1 and the previously characterized TNF2 (Bobe & Goetz 2001, Laing *et al.* 2001). Interestingly, TNFα mRNA expression has been reported in primary trout monocytes (Zou *et al.* 2002, MacKenzie *et al.* 2003) and in *in vitro* differentiated macrophages after stimulation with lipopolysaccharide (LPS) (MacKenzie *et al.* 2003). Furthermore, TNF-like activity has been found in supernatants of rainbow trout macrophages stimulated with LPS. These supernatants were able to enhance neutrophil migration and macrophage respiratory burst activity (Qin *et al.* 2001). These tools have been useful since, due to the lack of homologous TNFα peptides in fish, studies covering the biological functions of TNFα in these species have mainly been performed using mammalian recombinant TNFα or macrophage culture supernatants after being stimulated with LPS (Goetz *et al.* 2004). Recently, recombinant trout TNF1 and TNF2 proteins have been produced and they have been shown to induce gene expression of a number of proinflammatory factors in freshly isolated head kidney leucocytes and the macrophage cell line RST11. Furthermore, these proteins enhanced leucocyte migration and phagocytic activity *in vitro* in a dose-dependent manner (Zou *et al.* 2003).

In mammals, TNFα is secreted mainly by macrophages and monocytes but it can also be synthesized by other cell types. This cytokine plays an important role in the immune response and in inflammatory processes. However, TNFα has increasingly been recognized as a key

DOI: 10.1677/joe.1.05940
0022–0795/05/0184–527
Online version via http://www.endocrinology-journals.org

modulator of glucose homeostasis and lipid metabolism in adipose tissue (Sethi & Hotamisligil 1999). In fact, some authors have suggested that TNFα produced by the adipocyte itself acts as a true adipostat (Bulló-Bonet *et al.* 1999). Described effects of TNFα on lipid metabolism include stimulation of lipolysis in human adipocytes (Zhang *et al.* 2002), inhibition of the expression of enzymes involved in lipogenesis such as acetyl-CoA carboxylase and fatty acid synthase, and inhibition of lipoprotein lipase (LPL) activity (Semb *et al.* 1987, Grunfeld *et al.* 1989).

The mechanisms by which TNFα stimulates lipolysis are still largely unknown. In fact, TNFα is a potent activator of mitogen-activated protein kinases (MAPK), including ERK-1 and ERK-2 (p42/44), c-Jun NH_2-terminal kinase and p38 kinase (Wallach *et al.* 1999). In human adipocytes, Zhang *et al.* (2002) demonstrated that TNFα activated ERK and increased lipolysis, a mechanism which was blocked using two specific MEK inhibitors such as PD98059 and U0126. However, more research is needed in order to clarify the importance of the different MAPK pathways on the activation of lipolysis by TNFα.

Apart from the immune system, studies concerning the biological actions of TNFα in fish are scarce. However, some studies have already shown that TNFα could be a potentially important factor for immune–endocrine interactions in fish (Lister & van de Kraak 2002). In the present paper, we have investigated the role of TNFα in adipose tissue lipid metabolism in rainbow trout. To this end, we have examined the effects of TNFα in lipolysis on freshly isolated trout adipocytes using human recombinant TNFα (hrTNFα) and conditioned media from rainbow trout LPS-stimulated macrophages. Furthermore, in order to elucidate the mechanisms involved in TNFα-stimulated lipolysis, different MAPK inhibitors have been used. Finally, we studied the possible effects of LPS, a stimulus known to induce the expression of TNFα in trout macrophages (MacKenzie *et al.* 2003), administered *in vivo*, on lipolysis of rainbow trout adipocytes and LPL activity in trout adipose tissue. The data presented in this study suggest that TNFα plays an important role in lipid metabolism in trout by stimulating lipolysis *in vitro* and *in vivo* and by down-regulating LPL activity in adipose tissue *in vivo*.

Materials and Methods

Animals and experimental conditions

Mesenteric adipose tissue for *in vitro* studies was obtained from rainbow trout (*Oncorhynchus mykiss*) of an average weight of 266·45 ± 14·55 g, acclimated to laboratory conditions at the facilities of the University of Barcelona, Spain. Food, available *ad libitum*, was fed to animals by hand once a day and animals were acclimated for 10 days before the experiments were conducted. Fish were kept under natural conditions of light, latitude (40° 5′ N; 0° 10′ E) and temperature (15 ± 1°C).

For the LPS injection *in vivo* experiment, rainbow trout of an average weight of 154·89 ± 6·74 g were separated into two groups (12 fish in each group). On the day of the experiment, one group was injected intraperitoneally (i.p.) with LPS (6 mg/kg), a dose previously shown to induce the activation of the immune system in fish (MacKenzie *et al.* 2004, Ribas *et al.* 2004), and the other group was injected with saline (control group). Fish were anesthetized with 2-phenoxy-ethanol and immediately killed by a cranial blow at 24 and 72 h after the injection; blood was removed by caudal puncture using heparinized syringes. Immediately, fish were weighed and the adipose tissue was extracted to perform the adipocyte isolation. Furthermore, a portion of adipose tissue from each fish was separated and kept in liquid nitrogen for LPL activity analysis. At the end of the sampling, blood samples were centrifuged and different aliquots of plasma were kept at –20 °C until the day of analysis.

Experiments were conducted according to the Catalan government's 'Departament de Medi Ambient i Habitatge; Generalitat de Catalunya' regulations concerning treatment of experimental animals (No. 2215).

Adipocyte isolation

Adipocytes were isolated by the method of Robdell (1964) with some minor modifications. Fat tissue was cut into thin pieces and incubated for 60 min in polypropylene tubes with Krebs-Hepes buffer pre-gassed with 5% CO_2 in O_2 (pH 7·4) containing collagenase type II (130 U/ml) and 1% bovine albumin serum (BSA), in a shaking water bath at 15 °C. The cell suspension was filtered through a double layer of nylon cloth and then washed three times by flotation. Finally, cells were carefully resuspended in Krebs-Hepes buffer containing 2% BSA at a density of 6×10^5 cells/ml using a Fuchs-Rosenthal counting chamber. Aliquots of 400 μl of this final adipocyte suspension were incubated in polypropylene tubes in a shaking bath in the absence or presence of hrTNFα for up to 6 h at 15 °C. In a similar manner, adipocytes were incubated in the presence of hrTNFα and specific inhibitors of the MAPK pathway (PD98059 and SB203580). Adipocytes were pre-incubated for 20 min with the inhibitors before the addition of the cytokine.

At the end of the incubation time, tubes were rapidly placed on ice and cell-free aliquots of the medium were placed into enough perchloric acid to give a final concentration of 2%. Neutralized supernatants were taken for the measurement of glycerol concentration as an index of lipolysis using a spectophotometric method (Wieland 1984, Tebar *et al.* 1996). All products were obtained from Sigma Aldrich (Madrid, Spain). Control and experimental conditions were conducted in triplicate; results are the

average of triplicates from three independent experiments conducted with different adipocyte preparations. Previous experiments showed that basal lipolysis was proportional to cell density from 3×10^5 to 12×10^5 adipocytes/ml and also linear with incubation time for at least 7 h (Albalat *et al.* 2002).

Isolation of macrophages and production of TNF-containing supernatants

Rainbow trout macrophages were isolated from the head kidney and cultured as previously described (MacKenzie *et al.* 2003).

To obtain supernatants from LPS-activated macrophages, macrophages were incubated at a density of 1×10^7 cells/ml in DMEM high glucose medium (Life Technologies S.A., Spain) and stimulated with LPS (10 μg/ml) for 12 h at 18 °C under 5% CO_2. This concentration of LPS has previously been shown to be effective in stimulating TNFα expression in trout macrophages (MacKenzie *et al.* 2003). Following the incubation, the medium was collected and centrifuged for 10 min at 2000 ***g*** at 4 °C. Supernatants were pooled and 4 ml cell-free media were concentrated by filtration using Amicon Ultra-4 filters (Millipore Ibérica S.A., Madrid, Spain) to retain molecules with a molecular mass higher than 30 kDa (such as the trimeric form of TNFα, mass 51 kDa). The concentrated media were diluted (1/100) with Krebs buffer and used to incubate freshly prepared trout adipocytes. Adipocytes were incubated with LPS-stimulated media and non-stimulated media for 6 h at 15 °C in a shaking bath.

Lipoprotein lipase assay

A portion of adipose tissue from each fish was homogenized in 9 volumes homogenization buffer (10 mM HEPES, 1 mM EDTA and 1 mM dithiothreitol) at pH 7·4 and containing 5 U/ml heparin. Homogenates were centrifuged at 36 700 ***g*** at 4 °C for 20 min and the clear intermediate phase (between the fat droplets and the pellet) was used for the LPL activity assay. LPL activity was measured as previously described (Lindberg & Olivecrona 1995). LPL activity was performed with 10% Intralipid (Fresenius, Kabi, Spain) labeled with tri[9, 10-^{3}H]oleoglycerol by sonication in ice. The assay mixture (total volume 200 μl) contained 10 μl of the labeled Intralipid, 5% (vol/vol) pre-heated rat serum as an apo C-II source, 0·1 M NaCl, 0·15 M Tris–HCl, heparin (0·02%), bovine serum albumin (BSA, 60 mg/ml) and 25 μl of sample. After a 2-h incubation at 25 °C, the reaction was stopped by the addition of 2 ml isopropanol/heptane/1 M H_2SO_4 (40:48·3:1) and 0·5 ml water, and the free fatty acids were extracted as described (Bengtsson-Olivecrona & Olivecrona 1991). For each sample, four replicates were measured and activities were expressed as mU/g adipose tissue, considering that 1 mU is equivalent to 1 nmol of fatty acid released per min.

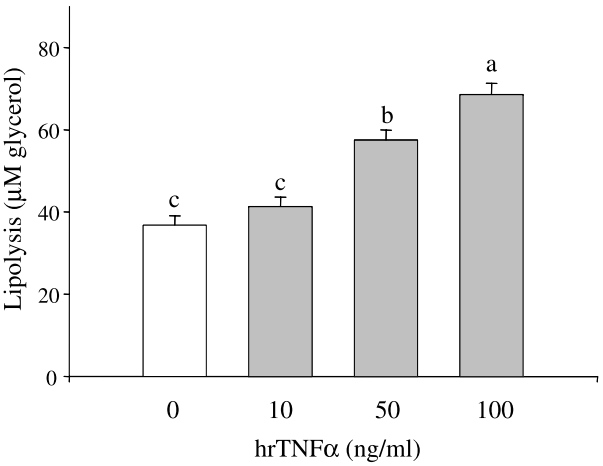

Figure 1 Effects of hrTNFα on lipolysis (measured as glycerol released in the medium) in rainbow trout isolated adipocytes after 6 h of incubation. Each point represents the average value ± S.E. of triplicates from 3 independent experiments. Values not sharing a common letter are significantly different ($P<0·05$; Tukey's test).

Insulin radioimmunoassay

Plasma insulin levels were measured by a radioimmunoassay (RIA) that used bonito insulin as standard and rabbit anti-bonito insulin antibodies as antiserum, according to the method of Gutiérrez *et al.* (1984).

Statistical analysis

All data are presented as means ± standard error (S.E.) ($n=6$ or 9 as stated). Results obtained were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test or by Student–Newman–Keuls test as indicated in the figures. Differences were considered significantly different when $P<0·05$.

Results

Dose–response of rhTNFα at different incubation times

hrTNFα was able to stimulate lipolysis of rainbow trout adipocytes in a dose-dependent manner after 6 h incubation beginning at 50 ng/ml, with a maximal stimulation of over twofold when adipocytes were incubated with hrTNFα at a concentration of 100 ng/ml (Fig. 1). Incubation of adipocytes with the highest concentration of TNF tested had no effects on the viability of the cells judged by cytological examination. Furthermore, lactate dehydrogenase (LDH) activity in the medium, commonly used as a marker of cell viability, was measured and LDH

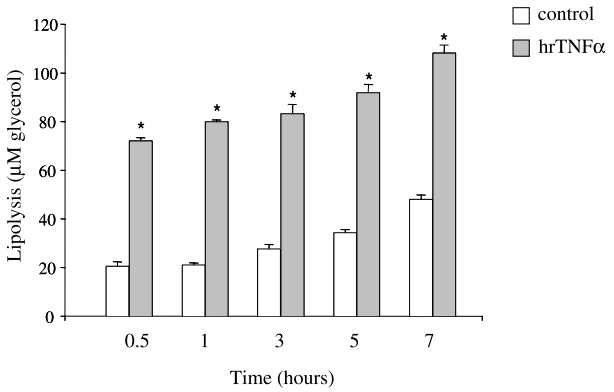

Figure 2 Time course of the effect of 50 ng/ml hrTNFα (shaded bars) on lipolysis in rainbow trout isolated adipocytes. Values are the average values ± S.E. of triplicates from a representative experiment from a total of 3 independent experiments. Values with an asterisk indicate significant differences between the corresponding control (open bars) ($P<0{\cdot}05$; Student–Neuman–Keuls test).

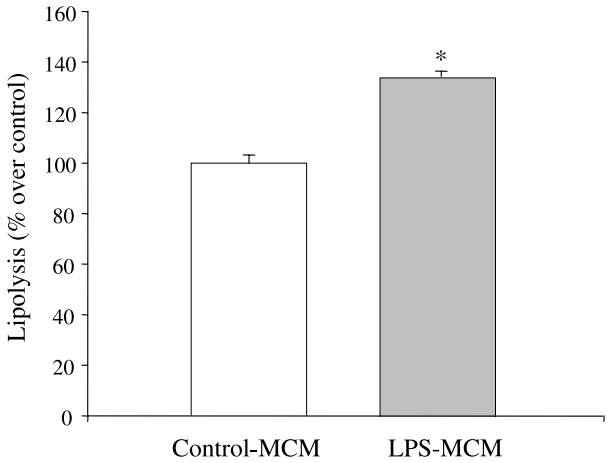

Figure 3 Effect of macrophage-conditioned media in rainbow trout adipocytes. Adipocytes were incubated with control macrophage-conditioned medium (control-MCM) and with LPS-stimulated macrophage conditioned-medium (LPS-MCM). Data are expressed as percent change with respect to the control group, which was set at 100% (43·92 ± 3·22 μM glycerol) and represent average values ± S.E. of triplicates from 2 independent experiments ($n=6$). The asterisk indicates a significant difference compared with the control ($P<0{\cdot}05$; Student–Neuman–Keuls test).

in the medium of control and TNF-exposed adipocytes was not significantly different, showing that TNFα did not affect viability of the cells after 6 h incubation (data not shown).

Time-course of rhTNFα on lipolysis in rainbow trout adipocytes

In order to study the time-course effects of hrTNFα on lipolysis in rainbow trout adipocytes, we used a concentration of 50 ng/ml rhTNFα. As shown in Fig. 2, rhTNFα at 50 ng/ml significantly ($P<0{\cdot}05$) stimulated glycerol release from adipocytes at times as short as 30 min and the stimulatory effect was maintained for up to 7 h of incubation time. However, since basal lipolysis (glycerol released in the medium in the absence of hormone) also increased with time, the maximum stimulation of rhTNFα was already obtained at 1 h of incubation.

Effect of conditioned medium from control and LPS-stimulated rainbow trout macrophages on lipolysis in rainbow trout adipocytes

Rainbow trout macrophages were incubated with control DMEM medium or with DMEM containing LPS (10 μg/ml) for 12 h. Conditioned media were concentrated and tested on freshly prepared isolated trout adipocytes for 6 h. As shown in Fig. 3, incubation of trout adipocytes with conditioned medium from LPS-stimulated macrophages (LPS-MCM) markedly stimulated lipolysis when compared with control-conditioned medium (control-MCM). Furthermore, the stimulation observed was due not to the presence of LPS but to other factors secreted in the medium by the macrophages since LPS added alone (10 μg/ml) did not significantly affect lipolysis in rainbow trout adipocytes incubated for the same period of time (control, 100 ± 6·17%; LPS, 90·45 ± 6·01% over control).

TNF-stimulated lipolysis is partially inhibited by MAPK inhibitors

To elucidate the signals involved in hrTNFα-induced lipolysis we used two specific inhibitors of the MAPK pathway, SB203580 for p38 and PD98059 for MEK1, the upstream kinase that activates p42/44. In these experiments, the adipocytes were pre-incubated with the inhibitors for 20 min before the addition of rhTNFα. As shown in Fig. 4, neither of the inhibitors at the highest concentration tested (50 μM) had any effect on basal lipolysis. Interestingly, SB203580 (Fig. 4A) had an inhibitory effect (at the two concentrations tested) on rhTNFα-stimulated lipolysis (50 ng/ml). However, this inhibitory effect was not observed when rhTNFα was added at a higher concentration (100 ng/ml). PD98059 (Fig. 4B) displayed a similar pattern since the highest concentration tested (50 μM) was able to partially inhibit the stimulatory effect of rhTNFα at 50 ng/ml but not at 100 ng/ml. Nevertheless, neither of the inhibitors tested was able to inhibit completely the stimulatory effects of rhTNFα under the conditions studied.

Effect of in vivo *LPS administration on rainbow trout adipocyte lipolysis*

When administered *in vivo*, LPS (6 mg/kg, i.p. injection) was able to stimulate basal *in vitro* lipolysis of rainbow trout

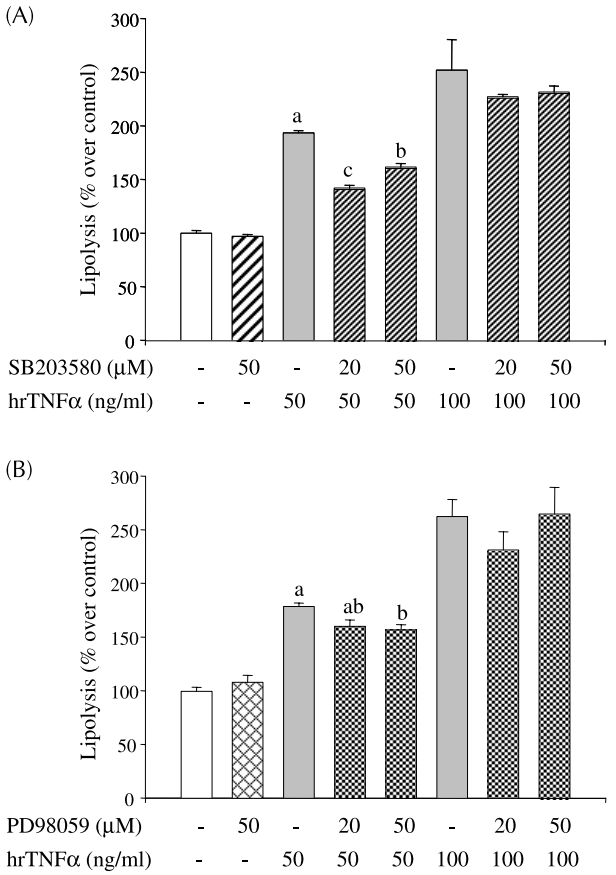

Figure 4 Effect of (A) SB203580 and (B) PD98059 on TNF-stimulated lipolysis. Inhibitors were pre-incubated for 20 min before the addition of the cytokine and adipocytes were incubated for 6 h. Data are expressed as percent change with respect to the control group, which was set at 100% (33·21 ± 1·63 and 30·35 ± 1·63 μM glycerol for SB203582 and PD98059 respectively) and represent average values ± S.E. of triplicates from 2 independent experiments (n=6) for each inhibitor used. Values not sharing a common letter are significantly different (P<0·05; Tukey's test).

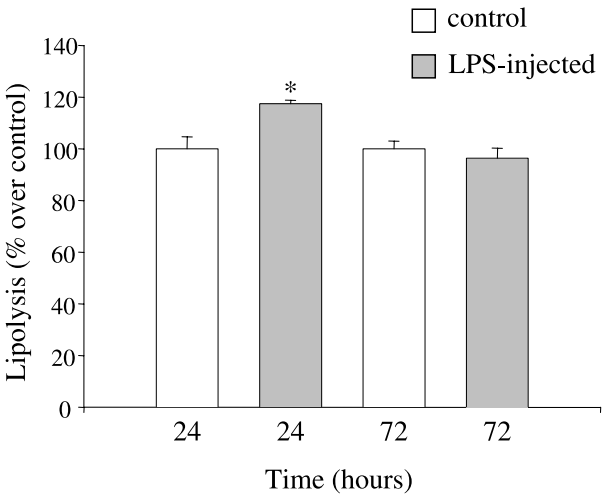

Figure 5 Effects of LPS administration *in vivo* on basal lipolysis in isolated adipocytes. Each point represents the average value ± S.E. from five replicates of samples from 6 fish per group. Data are expressed as percent change with respect to the control group, which was set at 100% (71·79 ± 3·34 and 72·54 ± 2·20 μM glycerol at 24 and 72 h respectively). An asterisk indicates a significant difference compared with the corresponding control (P<0·05; Student–Neuman–Keuls test).

adipocytes isolated 24 h after the injection, as shown in Fig. 5. This stimulation, however, was not observed 72 h after the injection. Furthermore, there was no significant difference in basal lipolysis in adipocytes from control (saline-injected) fish at 24 and 72 h after the injection.

In order to elucidate whether the LPS injection affected not only lipolysis in isolated adipocytes but also other mechanisms important for lipid metabolism, we measured LPL activity in adipose tissue at 24 h, which is when the LPS effect on lipolysis was clearly observed. LPL activity was significantly lower in adipose tissue from the LPS-injected group (99·79 ± 13·25 mU/g adipose tissue) when compared with the control-injected group (152·96 ± 10·10 mU/g adipose tissue). Since insulin is an important regulator of adipose LPL activity in mammals, we measured the levels of insulin in the plasma of animals used in the experiment at 24 h. However, plasma insulin levels were not significantly different between control and LPS-injected fish (4·75 ± 0·32 and 5·94 ± 0·32 ng/ml respectively). Therefore, the observed changes in adipose tissue lipid metabolism do not appear to be due to changes in plasma insulin levels.

Discussion

In the present study, we show that hrTNFα stimulates lipolysis in rainbow trout adipocytes in a dose-dependent manner. In fact, several studies have previously demonstrated that TNFα is able to increase the rate of lipolysis in different mammalian cell types such as 3T3-L1 adipocytes (Ogawa *et al.* 1989, Souza *et al.* 2003), human adipocytes (Zhang *et al.* 2002) and rat adipocytes (Gasic *et al.* 1999). However, to our knowledge, this is the first time that TNFα has been shown to stimulate lipolysis in fish adipocytes.

In our study, rhTNFα, at a concentration of 100 ng/ml, caused a twofold stimulation of lipolysis in rainbow trout adipocytes, which is comparable to the lipolytic response of human fat cells to rhTNFα (10–100 ng/ml) after a 48-h incubation (Rydén *et al.* 2002). In addition, other studies using mammalian adipocytes have demonstrated that maximal TNFα-stimulated lipolysis is obtained after 6–24 h of incubation (Hauner *et al.* 1995, Zhang *et al.* 2002). Nevertheless, because a mammalian peptide was used in the present experiments we cannot be sure that the

stimulation and time-course response would be identical with a homologous peptide. The apparent difference in the effective time to observe effects of TNFα between mammal and fish adipocytes (30 min) could be related to the existence of different intracellular activation mechanisms. In fact, the mechanism(s) by which TNFα stimulates lipolysis are not fully understood and they are strongly dependent on the cell type. In human preadipocytes, Rydén *et al.* (2002) found that TNFα-induced lipolysis involves the activation of the MEK1/2-ERK1/2 and JNK pathway but not the p38 pathway. Inhibitors of MEK1/2 and JNK, such as PD98059 and dimetylaminopurine, inhibited TNFα-induced lipolysis in human preadipocytes (Rydén *et al.* 2002). Similar results were obtained in 3T3-L1 adipocytes using inhibitors of the ERK pathway (Souza *et al.* 2003). In addition, Zhang *et al.* (2002) showed that PD98059 and U0126, another specific inhibitor of MEK1/2, inhibited not only TNFα-induced lipolysis but also basal lipolysis in human differentiated adipocytes. In the present study, we found that PD98059 did not have any effect on basal lipolysis but it partially blocked the lipolytic effects of rhTNFα in rainbow trout adipocytes. In a similar manner, SB203580 had no effect on basal lipolysis but was able to partially block rhTNFα-induced lipolysis in rainbow trout adipocytes. These observed effects of SB203580 differ from those observed in human preadipocytes where no effect of this inhibitor was observed on basal or TNFα-induced lipolysis (Rydén *et al.* 2002). However, in the present study neither of the inhibitors tested was able to completely block rhTNFα-mediated lipolysis. Therefore, we suggest that rhTNFα stimulates lipolysis in rainbow trout adipocytes, at least in part, through activation of ERK1/2 and p38 kinase.

The effects of rhTNFα on lipolysis in rainbow trout adipocytes were further confirmed by the results obtained with macrophage-conditioned media (MCM) in rainbow trout adipocytes. Trout adipocytes incubated in the presence of LPS-stimulated MCM had higher basal lipolysis than adipocytes incubated with control-MCM. The active factor(s) in LPS-stimulated MCM responsible for the induced lipolysis in trout adipocytes were not determined in this study. However, MacKenzie *et al.* (2003) have demonstrated that LPS increases the expression of TNFα mRNA in *in vitro* differentiated macrophages. Furthermore, supernatants harvested from trout macrophages stimulated with LPS exhibit TNF-like activities measured as enhanced neutrophil migration and enhanced macrophage respiratory burst activity (Qin *et al.* 2001). Outwith the immune system, Lister *et al.* (2002) found that trout MCM significantly inhibited human chorionic gonadotropin-stimulated testosterone production by goldfish (*Carassius auratus*) testis pieces *in vitro*, a typical feature described in mammals when cytokine levels are increased. In our study, LPS alone did not have any effect on basal lipolysis of rainbow trout adipocytes. Given these results, we suggest that at least one of the possible active factors for the observed induced lipolysis could be the presence of TNFα in LPS-stimulated MCM. However, since the active factor(s) responsible for the induced lipolysis were not determined, we should consider that it is possible that other cytokines, such as interleukin-1β, present in the LPS-stimulated supernatants could, in part, be responsible for the observed lipolytic effect.

Finally, we examined the effects of a single dose injection of LPS on rainbow trout adipocyte basal lipolysis. Adipocytes from LPS-injected fish had higher basal lipolysis compared with adipocytes from control fish 24 h after the LPS injection. Very few studies have checked the possible effects of an *in vivo* injection of LPS on adipocyte lipolysis, even in mammals. Pond & Mattacks (1998) observed an increase in basal lipolysis in guinea-pig adipocytes surrounding the popliteal lymph nodes after being activated with a subcutaneous injection of LPS. On the other hand, Porter *et al.* (2002) showed that exposure of adipose tissue explants to TNFα for 24 h produced an increase in adipocyte glycerol release in a short-term incubation.

In addition to the effects on lipolysis, we found that *in vivo* treatment with LPS inhibited LPL activity in rainbow trout adipose tissue at 24 h. LPL is a key enzyme that provides fatty acids from triglycerides to peripheral tissues such as adipose tissue (Enerbäck & Gimble 1993). In mammals, it is clearly recognised that there are mainly two situations where adipose tissue LPL is down-regulated. One is food deprivation and the other is trauma/sepsis/LPS administration, which is a response primarily mediated by TNFα (Wu *et al.* 2004). The results obtained in our study are in agreement with several studies which reported that LPL activity is inhibited by TNFα in mammalian adipose tissue (Semb *et al.* 1987, Grunfeld *et al.* 1989) and with the results presented by Kawasaki *et al.* (2004) where an LPS injection in rats provoked a decrease in LPL activity in adipose tissue and an increase in hormonal sensitive lipase 2 h after the LPS injection. Importantly, the decrease observed in LPL activity by LPS administration in the present study could not be related to changes in plasma insulin levels, since similar insulin levels were found in the saline- and LPS-injected groups. Nevertheless, from our data it is not possible to conclude that insulin did not affect LPL activity since insulin was only measured at one sampling time (24 h). More data on plasma insulin levels between the injection and the 24-h time point would be necessary in order to clarify a possible role of insulin in the observed effect in LPL activity. Moreover, future studies should investigate whether hrTNFα has direct effects on LPL activity in isolated adipocytes.

In summary, hrTNFα stimulated lipolysis in isolated rainbow trout adipocytes by a signaling mechanism that involved, at least in part, activation of ERK1/2 and p38 kinase. Moreover, conditioned medium from LPS-stimulated trout macrophages was able to induce an

increase in the lipolytic rate in isolated rainbow trout adipocytes. Finally, *in vivo* LPS administration induced an increase in lipolysis in isolated rainbow trout adipocytes and a down-regulation of LPL activity in rainbow trout adipose tissue 24 h after the injection, suggesting that several mechanisms important for lipid metabolism are altered due to the administration of LPS. We suggest that the *in vivo* effects of LPS on lipid metabolism are probably mediated by TNFα, which could be secreted by macrophages or by adipose tissue itself (Sewter *et al.* 1999, MacKenzie *et al.* 2003).

To our knowledge, this is the first time that the effects of TNFα have been evaluated in fish adipocytes. Although more research is needed, we suggest that TNFα could be a key modulator of lipid metabolism in fish and that the metabolic activity of TNFα has been conserved during the evolution from fish to mammals.

Acknowledgements

We thank J Baró from the fish farm Truites del Segre (Lleida) for providing the rainbow trout and J Guinea from the Estabulari de la Facultat de Biologia for the maintenance of the fish.

Funding

This study was supported by grants from the Centre de Referància de Recerca i Desenvolupament en Aqüicultura to I N and L T. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Received 29 July 2004
Accepted 23 November 2004