General and Comparative Endocrinology 228 (2016) 1–8

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

# Tumour necrosis factor-α inhibits hepatic lipid deposition through GSK-3β/β-catenin signaling in juvenile turbot (*Scophthalmus maximus* L.)

Dongwu Liu, Kangsen Mai, Yanjiao Zhang, Wei Xu, Qinghui Ai *

*Key Laboratory of Aquaculture Nutrition and Feed (Ministry of Agriculture) and the Key Laboratory of Mariculture (Education Ministry of China), Ocean University of China, Qingdao 266003, PR China*

ARTICLE INFO

*Article history:*
Received 9 September 2015
Revised 14 December 2015
Accepted 29 December 2015
Available online 30 December 2015

*Keywords:*
Tumour necrosis factor-α
Lipid deposition
GSK-3β
β-Catenin
Liver
Juvenile turbot

ABSTRACT

In this study, the mechanism that TNFα inhibits lipid deposition through GSK-3β/β-catenin signaling was investigated in the liver of juvenile turbot (*Scophthalmus maximus* L.) by injection of TNFα or TNFα inhibitor pomalidomide (POM). It was found that TNFα inhibited the expression of GSK-3β and induced β-catenin expression. TNFα inhibited the expression of peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer binding protein α (C/EBPα), as well as the activity of lipoprotein lipase (LPL) and fatty acid synthetase (FAS). In addition, the level of triglyceride (TG), total cholesterol (TC), non-esterified fatty acid (NEFA), and glycerol was decreased by TNFα treatment in the liver. In the plasma, the level of TG, TC, low density lipoprotein cholesterol (LDL-C), NEFA, and glycerol was decreased, but high density lipoprotein cholesterol (HDL-C) was increased by TNFα treatment. However, compared to TNFα, POM had the opposite effect on the biochemical indexes and genes related to lipid deposition in the liver. The results indicated that TNFα may regulate hepatic lipid metabolism and fat distribution through GSK-3β/β-catenin signaling as well as transcription factors PPARγ and C/EBPα in juvenile turbot.


## 1. Introduction

As one of the important adipokines secreted from adipose tissue, tumor necrosis factor alpha (TNFα) is a negative regulator of insulin signal transduction (Ahima and Flier, 2000; Weisberg et al., 2003). TNFα is involved in regulating insulin resistance, obesity, as well as fatty acid (FA) and glucose metabolism. It has been confirmed that both adipose tissue and macrophages from activated adipose tissue secret TNFα (Weisberg et al., 2003; Hotamisligil et al., 1993). The regulatory mechanisms of TNFα on adipose tissue have been fully elucidated in mammals in the past decades (Sethi and Hotamisligil, 1999; Warne, 2003; Langin and Arner, 2006; Cawthorn and Sethi, 2008). In addition, TNFα is a limiting factor of lipid deposition in some fish species (Saera-Vila et al., 2007; Cruz-Garcia et al., 2009). TNFα participates in regulating fat tissue mass in the gilthead sea bream (*Sparus aurata* L.) (Saera-Vila et al., 2007), controlling adipose tissue lipid metabolism, and inhibiting the differentiation of adipocyte precursor cells in rainbow trout (*Oncorhynchus mykiss*) (Albalat et al., 2005; Bouraoui et al., 2008).

In the canonical Wnt/β-catenin signaling pathway, β-catenin plays a central role as a transcriptional coactivator. When the ligands of Wnt bind to frizzled/low density lipoprotein receptor related protein (LRP) receptor complex, β-catenin is hypophosphorylated, translocates into the nucleus, and further induces the expression of target genes (Cossu and Borello, 1999; Ridgeway et al., 2000). As an important physiological regulator of mammalian hepatic metabolism, Wnt signaling affects the hepatic glucose, glycogen, and lipid metabolism (Liu et al., 2011). Wnt/β-catenin signaling interacts with the peroxisome proliferator-activated receptor γ (PPARγ) (Liu et al., 2006), and regulates hepatic FA metabolism as well as the hepatic mitochondrial function, energy balance, and FA oxidation (Gebhardt and Hovhannisyan, 2010; Lehwald et al., 2012). In addition, Wnt/β-catenin signaling is involved in the alteration in cholesterol metabolism and perivenous accumulation of triglycerides (TGs) in APC-deficient mice (Mutoh et al., 2006). The inhibition of Wnt signaling decreases the body fat mass and hepatic gluconeogenesis, but increases the brown adipose tissue and hepatic sensitivity to insulin (Liu et al., 2012).

Juvenile turbot (*Scophthalmus maximus* L.) is an economically important marine flatfish species, and the liver is one of the principal sites of lipid storage. For the regulatory mechanism of TNFα on fish hepatic lipid deposition remains largely unknown, it is essential to investigate whether GSK-3β/β-catenin signaling is involved in this regulatory process. In previous studies, the recombinant human TNFα is successfully used as a lipid metabolism

* Corresponding author.
*E-mail address:* qhai@ouc.edu.cn (Q. Ai).

http://dx.doi.org/10.1016/j.ygcen.2015.12.027
0016-6480/

regulator in fish (Albalat et al., 2005; Cruz-Garcia et al., 2009; Vraskou et al., 2011; Sánchez-Gurmaches et al., 2012). Moreover, pomalidomide (POM) is a novel immunomodulatory drug and effectively inhibits TNFα secretion (Corral et al., 1999; Tefferi et al., 2009). In this study, the mechanism that TNFα regulates lipid deposition through GSK-3β/β-catenin signaling was investigated in the liver of juvenile turbot (*S. maximus* L.) by injection of TNFα or TNFα inhibitor POM. Moreover, the effect of TNFα on the key enzymes, hepatic biochemical indexes, and transcriptional factors related to lipid metabolism was analyzed in the juvenile turbot.

## 2. Materials and methods

### 2.1. Animals and experimental conditions

Juvenile turbot (*S. maximus* L.) were purchased from a commercial farm (Qingdao, China) and kept in an indoor circulatory system in the experimental station of the Ocean University of China. Fish of similar sizes (average weight 50.32 ± 0.15 g) were randomly distributed into nine 400-L circular fiberglass flat-bottom tanks at a density of 15 fish per tank. Animals were fed ad libitum once daily with a commercial diet (Qihao Biotech Co. Shandong, China) and acclimated for 15 days. Fish were reared under the following conditions: water temperature, 17.5–18.5 °C; salinity, 30–32‰; dissolved oxygen, 7 mg/L; photoperiod, 12:12 h (dark:light); ammonia–nitrogen and nitrite, lower than 0.1 mg/L. The animal procedures were approved by Ocean University of China's Institutional Animal Care Committee in accordance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of China).

### 2.2. Injection experiment

Animals were injected intraperitoneally with 0.5 μg/ml recombinant human TNFα (1.0 ng/g body mass), 2.0 mg/ml POM (4.0 μg/g body mass), or 0.9% sodium chloride (2 μl/g body weight) two times a week, and the injection was conducted for two weeks. TNFα was purchased from GenScript Corporation (USA) and POM was purchased from MedChem Express Corporation (USA). TNFα or POM was dissolved in 0.9% sodium chloride, respectively. The animals injected with sodium chloride were treated as the control. Triplicate tanks were used for fish treated with saline, TNFα, or POM, respectively.

### 2.3. Sample collection

After the injection experiment, the livers from five fish were removed and pooled for biochemical analysis, and the livers from two fish were removed for the analysis of gene and protein expression in each tank. Subsequently, blood samples from another three fish in each tank were taken from the caudal vein using heparinized syringes to obtain plasma after centrifugation for 10 min at 1400×*g* (4 °C). The plasma and liver samples were immediately frozen in liquid nitrogen and then stored at −80 °C until analysis. For oil red O staining, the livers were cut and fixed for 24 h in a solution of 4% paraformaldehyde dissolved in 0.1 mol/L sodium phosphate buffer (PBS, pH7.4, 4 °C).

### 2.4. Biochemical analysis

Liver samples in each tank were homogenized in ice-cold 0.7% saltwater, and the supernatants were collected for biochemical analysis after centrifugation at 3000 r/min for 10 min. The level of triglyceride (TG), total cholesterol (TC), nonesterified fatty acid (NEFA), glycerol, LPL activity, FAS activity, as well as the protein concentration of liver supernatants were measured according to the analytical procedures specified in the commercially available kits (Nanjing Jiancheng Bioengineering Institute, China). Furthermore, the levels of TG, TC, NEFA, glycerol, low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) in plasma were measured according to the analytical procedures specified in the commercially available kits (Nanjing Jiancheng Bioengineering Institute, China).

### 2.5. Oil red O staining

The livers were washed three times with PBS (pH 7.4), sectioned at a thickness of 20 μm in a cryostat (Leica CM1850, Germany), and mounted on chromalum-gelatin-coated glass slides. The tissues were stained with oil red O kit following standard procedures specified in the kit (Nanjing Jiancheng Bioengineering Institute, China). The slides were re-dyed with hematoxylin and analyzed under light microscope. Six images were randomly selected to count the number of lipid droplets.

### 2.6. RNA extraction and real-time quantitative polymerase chain reaction

Total RNA was extracted from livers using Trizol reagent (Invitrogen, USA) and the integrity was tested. Then cDNA was synthesized with EasyScript First-Strand cDNA Synthesis SuperMix kit (TransGen, Beijing, China). The primer sequences of GSK-3β, β-catenin, PPARγ, CEBPα, LPL, FAS, and β-actin were designed following the published sequences from turbot (Table 1). SYBR® Premix Ex Taq™ II (TransGen, Beijing, China) was used to quantify genes expression with a quantitative thermal cycle (Mastercycler® ep realplex; Eppendorf, Germany). The real-time quantitative PCR program was as follows: 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 20 s. The reaction was carried out with three duplicates for each sample, and the gene expression levels were calculated according to the method of Livak and Schmittgen (2001).

### 2.7. Western blot analysis

Frozen livers were homogenized on ice with a homogenizer in 2 ml of buffer containing 150 mmol/L NaCl, 10 mmol/L Tris, 1 mmol/L EGTA, 1 mmol/L EDTA (pH 7.4), 100 mmol/L NaF, 4 mmol/L sodium pyrophosphate, 2 mmol/L sodium orthovanadate, 1% Nonidet P-40, and a protease inhibitor cocktail (Roche, USA). Western blot was carried out with the standard procedures. Briefly, homogenates were centrifuged at 20,000*g* at 4 °C for 30 min. Protein concentrations were determined using a protein assay kit (Nanjing Jiancheng Bioengineering Institute, China), and lysates were subjected to SDS–PAGE and western blotting. After blocked with TBST (25 mM Tris, pH 7.4, 150 mM NaCl and 0.1% Tween-20) containing 5% nonfat dry milk, nitrocellulose membranes were incubated with rabbit polyclonal antibody anti-β-catenin (sc-1496, Santa-Cruz, 1:500), goat polyclonal antibody anti-GSK-3β (sc-9166, Santa-Cruz, 1:500), goat polyclonal antibody anti-PPARγ (sc-1984, Santa-Cruz, 1:500), goat polyclonal antibody anti-C/EBPα (sc-9314, Santa-Cruz, 1:500), and goat polyclonal antibody β-actin (sc-130657, Santa-Cruz, 1:1000). According to our previous study, the antibodies could cross-react with turbot proteins (Liu et al., 2016). Then HRP-conjugated secondary antibody (Santa-Cruz, 1:3000) was incubated and visualized by chemiluminescence using an ECL detection kit (Nanjing Jiancheng Bioengineering Institute, China). Finally, the optical density of protein bands was evaluated using Image J software (NIH Image, Bethesda, MD, USA).

**Table 1**
Real-time quantitative PCR primers for genes related to lipid deposition and β-actin of juvenile turbot.

| Target gene | Forward (5′-3′) | Reverse (5′-3′) | Size (bp) | GenBank |
|---|---|---|---|---|
| GSK-3β | CTGGCCTACATCCACTCGTT | CACTGCCAAAGTCACAGAGC | 106 | KT372085 |
| β-Catenin | TCCACAACAGAATCGTCATCA | CAGTTCACACAGCACACCAG | 113 | KT372083 |
| PPARγ | AAGTGACGGAGTTCGCCAAGA | GTTCATCAGAGGTGCCATCA | 121 | 1694842 |
| CEBPα | ACGAGACCTCCATCGAACTG | CATGATCTTCAGCTTGTCCTG | 110 | DQ848934 |
| LPL | CTCCCACGAACGCTCTAT | GCGGACCTTGTTGATGTT | 166 | JQ690822 |
| FAS | GGCAACAACACGGATGGATAC | CTCGCTTTGATTGACAGAACAC | 205 | KC189927 |
| β-Actin | GTAGGTGATGAAGCCCAGAGCA | CTGGGTCATCTTCTCCCTGT | 204 | AY008305 |

### 2.8. Statistical analysis

The results of gene expression were expressed as means ± standard error of mean (s.e.m) ($n = 6$ fish). The data for enzyme activity, biochemical indexes, and western blot were expressed as means ± s.e.m ($n = 3$ tanks). The statistical analyses were performed using SPSS 17.0 (SPSS Inc., USA). The normality and homogeneity of variances among groups were tested, and results were subjected to one way ANOVA. Duncan's test for multiple comparisons of means was applied. Differences were considered significant at a level of 95% ($P < 0.05$).

## 3. Results

### 3.1. Effect of TNFα and POM on GSK-3β and β-catenin expression

To elucidate whether TNFα affects GSK-3β/β-catenin signaling in juvenile turbot, fish were injected with TNFα or POM. TNFα significantly inhibited the mRNA and protein expression of GSK-3β, while POM significantly induced the mRNA and protein expression of GSK-3β (Figs. 1A and 2A). However, the mRNA and protein expression of β-catenin was significantly induced by TNFα, but significantly inhibited by POM (Figs. 1B and 2B).

### 3.2. Effect of TNFα and POM on PPARγ and C/EBPα expression

The mRNA expression of PPARγ and C/EBPα was significantly inhibited by TNFα, but significantly induced by POM treatment (Fig. 1C and D). Moreover, the protein expression of PPARγ and C/EBPα was significantly inhibited by TNFα, while significantly induced by POM treatment (Fig. 2C and D).

### 3.3. Effect of TNFα and POM on gene expression and activity of enzymes related to lipogenesis

After TNFα treatment, a clear reduction in LPL and FAS mRNA expression was observed (Fig. 1E and F). Nevertheless, LPL and FAS mRNA expression was significantly induced by POM treatment (Fig. 1E and F). Moreover, TNFα significantly inhibited LPL and FAS activity, while POM significantly induced LPL and FAS activity (Fig. 3A and B). In addition, compared to the control, there were fewer lipid droplets in the liver of TNFα treatment (Fig. 4A–D).

### 3.4. Effect of TNFα and POM on the biochemical indexes in liver and plasma

In the liver of juvenile turbot, the level of TG, TC, NEFA, and glycerol was significantly decreased by TNFα treatment, but significantly increased by POM treatment (Fig. 5A–D). In the plasma, the level of TG, TC, LDL-C, NEFA, and glycerol was significantly decreased by TNFα treatment, but significantly increased by POM treatment (Fig. 6A, B, D, E, F). However, the level of HDL-C was significantly increased by TNFα, but significantly decreased by POM (Fig. 6C).

## 4. Discussion

The key event in Wnt/β-catenin signaling is the cytoplasmic β-catenin accumulation and its subsequent nuclear translocation. In this process, GSK-3β plays a significant role in regulating β-catenin accumulation (Aberle et al., 1997; Huelsken and Behrens, 2002). In this study, TNFα significantly inhibited GSK-3β expression and induced β-catenin accumulation in the liver of juvenile turbot. However, compared to TNFα, TNFα inhibitor POM had the inverse effect on the expression of GSK-3β and β-catenin. It has been found that TNFα promoted nuclear β-catenin accumulation through inhibiting GSK-3β activity in the gastric tumour cells (Oguma et al., 2008). For the level of β-catenin is closely associated with the activity of GSK-3β, TNFα may induce β-catenin accumulation by inhibiting GSK-3β expression in the liver of juvenile turbot.

The development of adipose tissue includes the hypertrophy of existing adipocytes and the proliferation of new ones. In 3T3-L1 preadipocytes, β-catenin signaling inhibits adipocyte differentiation through inhibiting C/EBPα and PPARγ expression (Moldes et al., 2003; Ross et al., 2000; Luo et al., 2009). Moreover, TNFα inhibits adipogenesis of 3T3-L1 preadipocytes by preventing the early induction of PPARγ and C/EBPα via β-catenin signaling (Cawthorn et al., 2007). Three PPARs have been characterized in the sea bass (*Dicentrarchus labrax*), and PPARγ plays an important role in the peroxisomal β-oxidation of fatty acids in salmon (Boukouvala et al., 2004; Ruyter et al., 1997). In this study, the expression of PPARγ and C/EBPα was inhibited by TNFα, which is consistent with the previous studies. Since TNFα significantly induced the expression of β-catenin, TNFα may inhibit the expression of PPARγ and C/EBPα through β-catenin signaling in the liver of juvenile turbot.

In fish, liver is one of the main sites for lipid synthesis and storage (Robinson and Mead, 1973; Sheridan, 1988). There are various enzymes involved in the hepatic lipid deposition, in which FAS and LPL play a significant role (Richard et al., 2006; Nilsson-Ehle et al., 1980). LPL hydrolyzes TGs in plasma lipoproteins and supplies free FAs for storage or oxidation (Nilsson-Ehle et al., 1980). In the previous studies, TNFα reduces the expression of LPL, limits the uptake of FAs, and ultimately increases tissue lipid reservoirs in gilthead sea bream (Saera-Vila et al., 2007). Moreover, TNFα plays an important role in the control of lipid metabolism in rainbow trout by stimulating lipolysis *in vitro* and *in vivo* (Albalat et al., 2005). In this study, TNFα inhibits the expression and activity of LPL and FAS in the liver of juvenile turbot. The level of TG, TC, NEFA, and glycerol was decreased by TNFα in the liver. In the plasma, the level of NEFA, glycerol, TG, TC, and LDL-C was decreased, but HDL-C was increased by TNFα. The results indicated that TNFα participated in the hepatic lipid metabolism by regulating the activity of FAS and LPL.

Wnt/β-catenin plays an important role in maintaining liver health and pathophysiology (Thompson and Monga, 2007). This signaling was critically involved in de novo lipogenesis and adipogenesis in nonalcoholic fatty liver disease (Song et al., 2014). Another study demonstrated that LRP5-driven β-catenin signaling

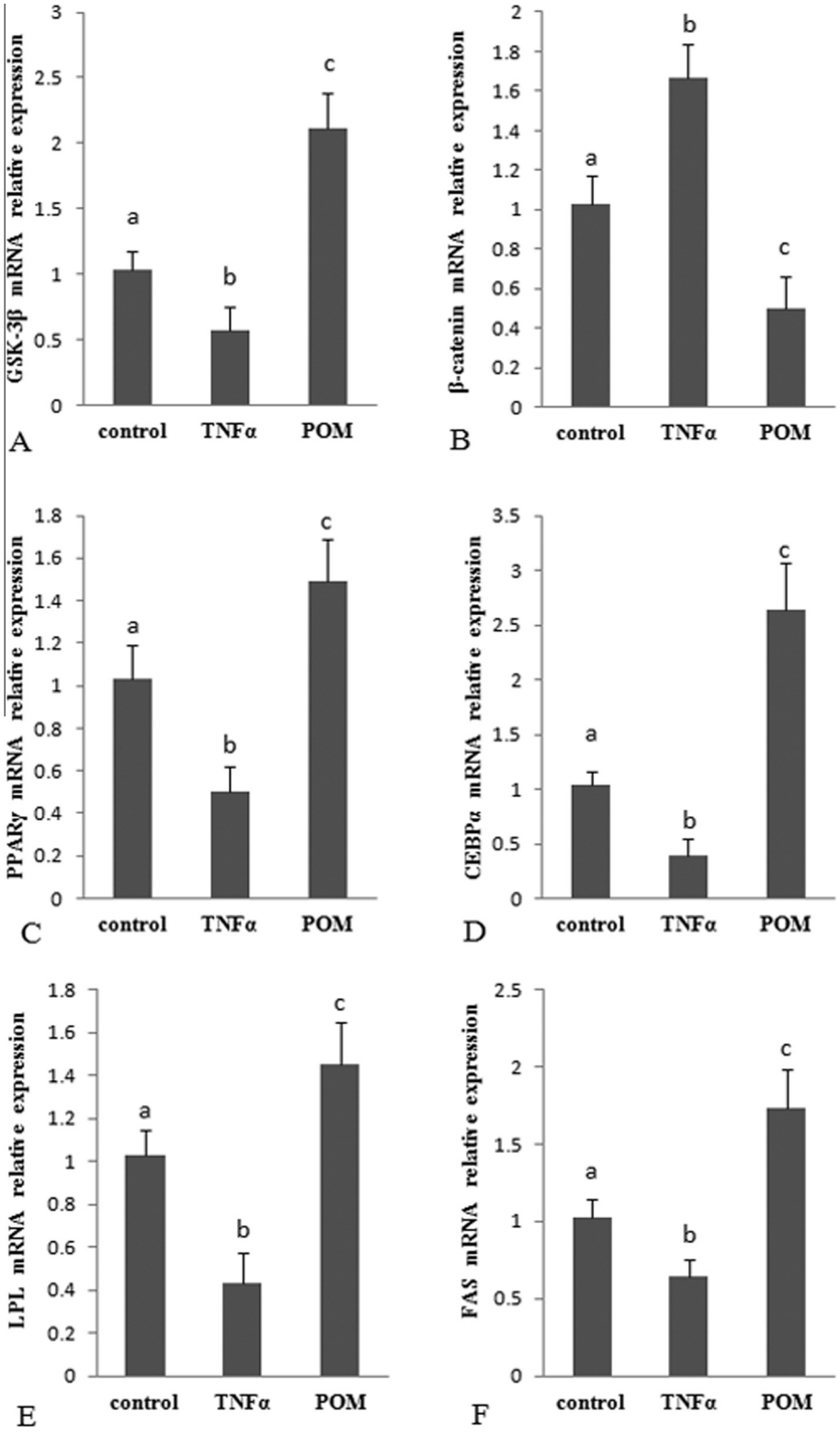


**Fig. 1.** Effect of TNFα and POM on the mRNA expression of genes related to lipid deposition in the liver of juvenile turbot. (A) GSK-3β; (B) β-catenin; (C) PPARγ; (D) C/EBPα; (E) LPL; (F) FAS. Values are expressed as means ± s.e.m. ($n$ = 6 fish). Statistically significant differences are denoted by different letters.

regulated adipose progenitor proliferation and differentiation in a dose- and depot-specific manner, and further modulated human body fat distribution (Loh et al., 2015). Furthermore, the inhibition of Wnt signaling decreases the body fat mass and hepatic gluconeogenesis, and increases the brown adipose tissue and hepatic sensitivity to insulin (Liu et al., 2012). In our previous study, Wnt/β-catenin signaling participates in the regulation of lipogenesis in the juvenile turbot (Liu et al., 2016). For TNFα activates GSK-3β/β-catenin signaling and affects the biochemical indexes in liver and plasma, TNFα may regulate hepatic lipid metabolism and fat distribution through GSK-3β/β-catenin signaling in juvenile turbot. In addition, liver steatosis is associated with nutritional imbalances

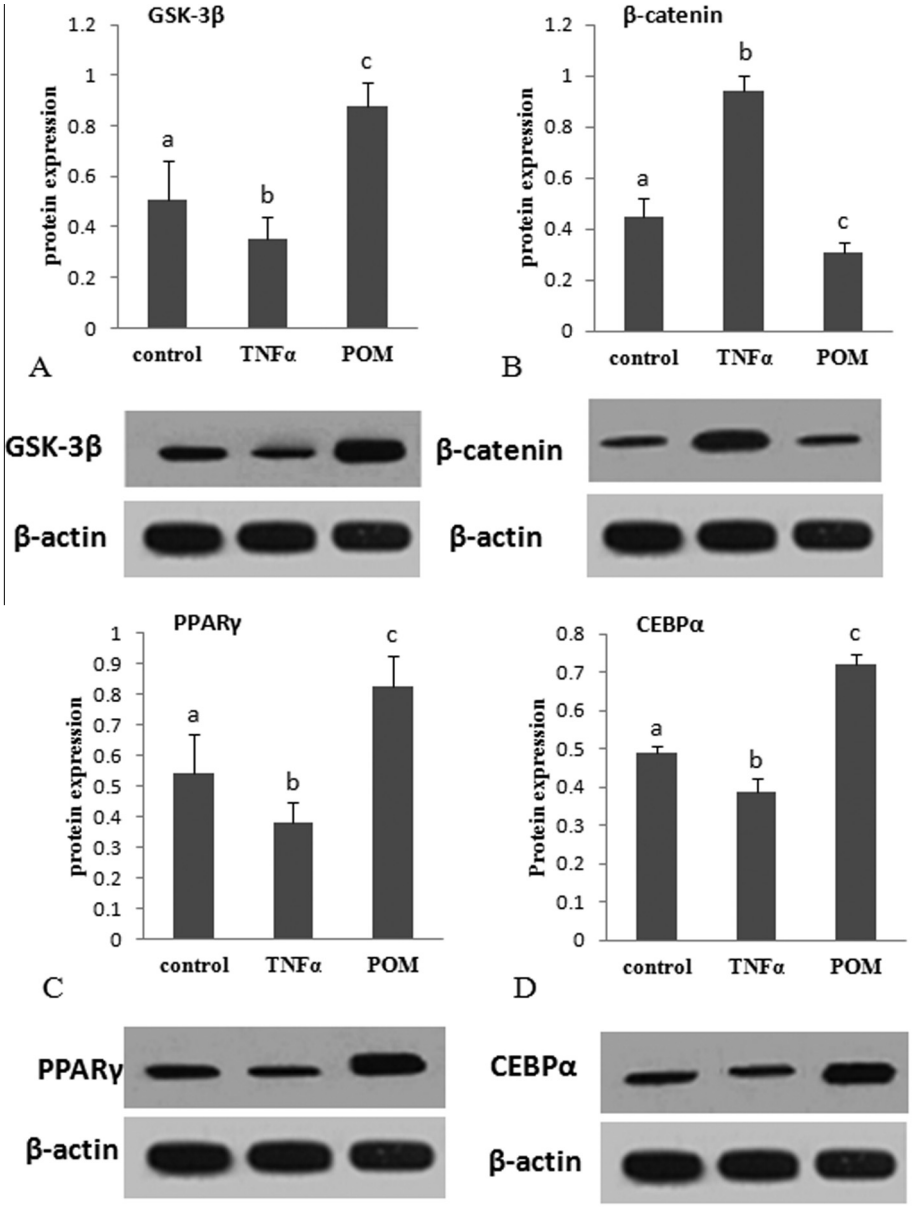


**Fig. 2.** Effect of TNFα and POM on the protein expression of GSK-3β, β-catenin, PPARγ, and C/EBPα in the liver of juvenile turbot. (A) GSK-3β; (B) β-catenin; (C) PPARγ; (D) C/EBPα. Values are expressed as means ± s.e.m. ($n$ = 3 tanks). Statistically significant differences are denoted by different letters.

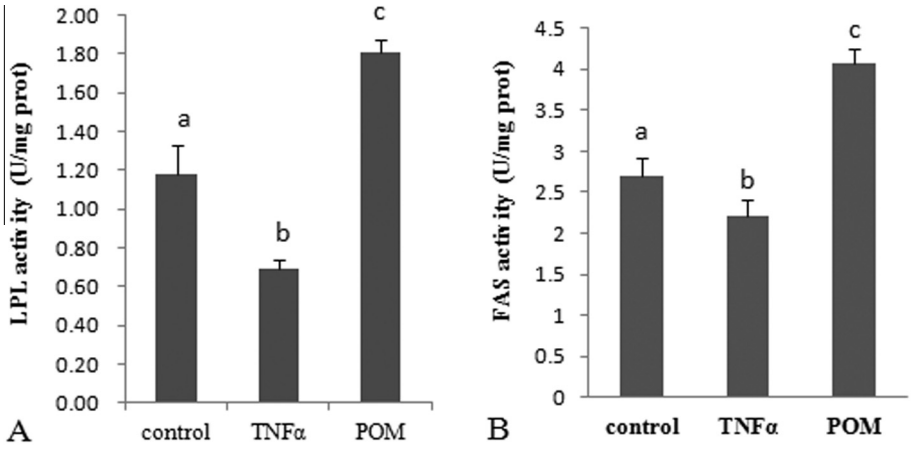


**Fig. 3.** Effect of TNFα and POM on the activity of LPL and FAS in the liver of juvenile turbot. (A) LPL activity; (B) FAS activity. Values are expressed as means ± s.e.m. ($n$ = 3 tanks). Statistically significant differences are denoted by different letters.

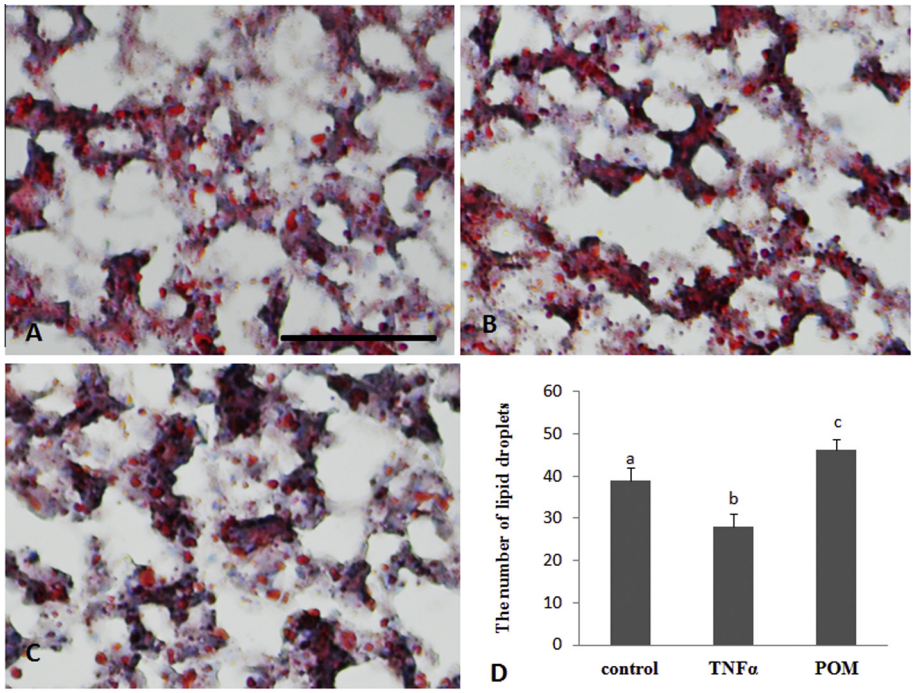


**Fig. 4.** Effect of TNFα and POM on the lipid droplets in the liver of juvenile turbot. (A) Control; (B) TNFα treatment; (C) POM treatment; (D) the number of lipid droplets. Bar: 50 μm.

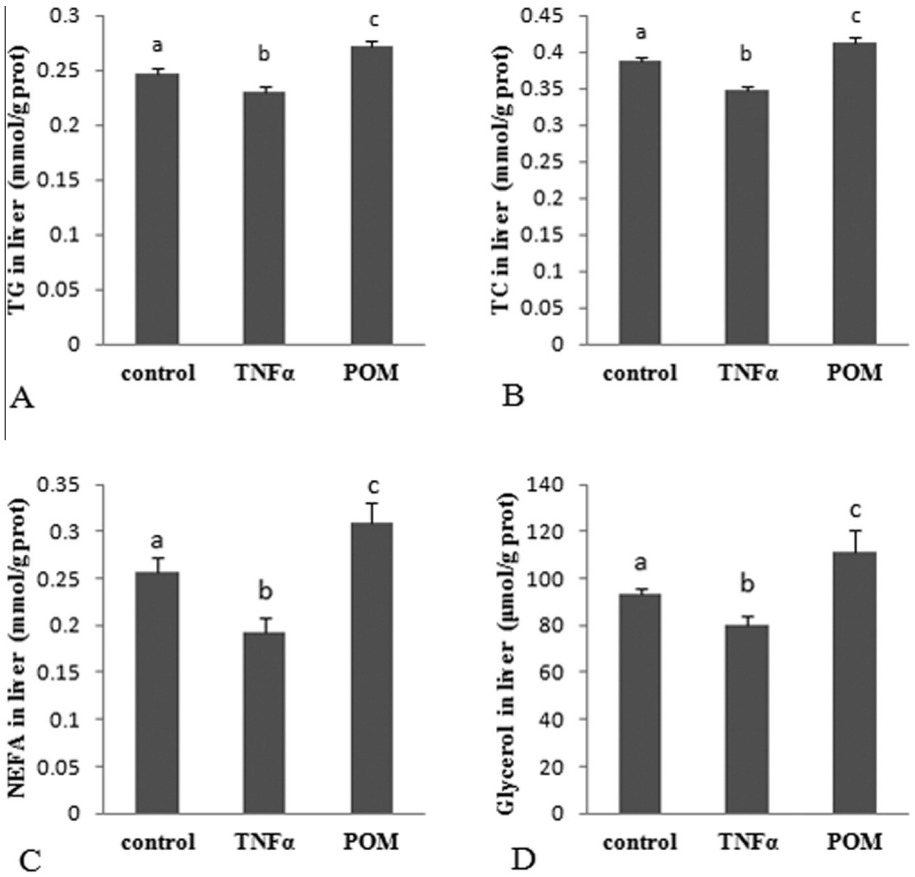


**Fig. 5.** Effect of TNFα and POM on biochemical indexes in the liver of juvenile turbot. (A) TG; (B) TC; (C) NEFA; (D) glycerol. Values are expressed as means ± s.e.m. ($n$ = 3 tanks). Statistically significant differences are denoted by different letters.

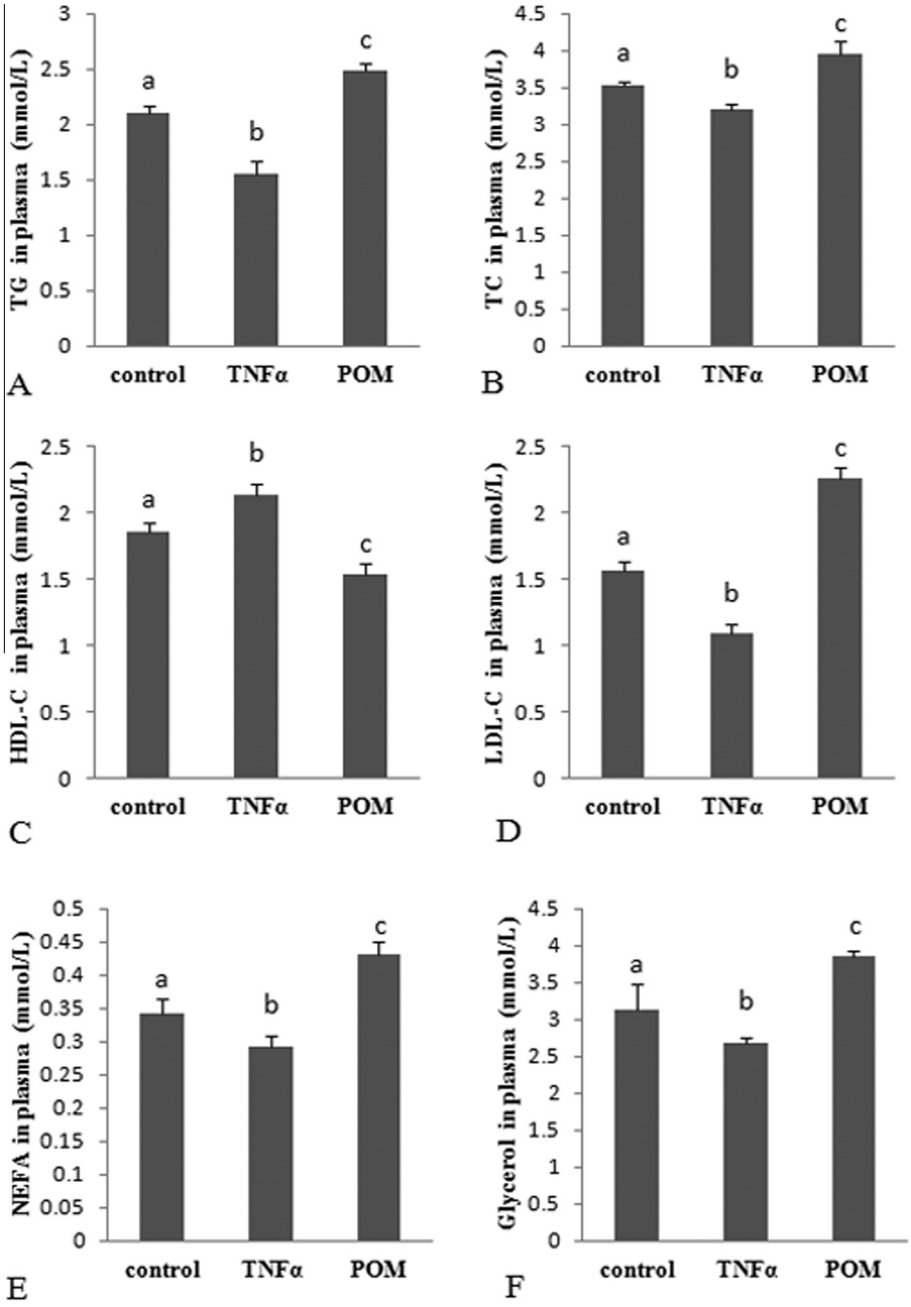


**Fig. 6.** Effect of TNFα and POM on biochemical indexes in the plasma of juvenile turbot. (A) TG; (B) TC; (C) HDL-C; (D) LDL-C; (E) NEFA; (F) glycerol. Values are expressed as means ± s.e.m. ($n$ = 3 tanks). Statistically significant differences are denoted by different letters.

in cultured fish (Tacon, 1996). High lipid intake elicits the induction of fatty liver syndrome and abnormal oxidative status (Dos Santos et al., 1993; Du et al., 2006; Gao et al., 2011). Since TNFα inhibits hepatic lipid distribution through GSK-3β/β-catenin signaling, TNFα may be supplemented as a lipid regulator in fish diets.

In summary, the mechanism that TNFα inhibits lipid deposition through GSK-3β/β-catenin signaling was investigated in the liver of juvenile turbot (*S. maximus* L.) by TNFα or POM treatment. The results indicated that GSK-3β/β-catenin signaling was activated by TNFα treatment. TNFα inhibited the activity of LPL and FAS, and regulated the biochemical indexes in the liver and plasma. In addition, the expression of PPARγ and C/EBPα was inhibited by TNFα treatment. TNFα may regulate hepatic lipid metabolism and fat distribution through GSK-3β/β-catenin signaling as well as transcription factors PPARγ and C/EBPα in juvenile turbot. This regulatory mechanism is demonstrated for the first time in fish species, which may be a valuable contribution to understanding the modulatory mechanism of TNFα on hepatic lipid deposition.

## Acknowledgments

This work was supported by National Basic Research Program of China (2014CB138600), the China Agriculture Research System (CARS-50-G08), and the Doctoral Scientific Fund Project of the Ministry of Education of China (20120132110007).

## References

Aberle, H., Bauer, A., Stappert, J., Kispert, A., Kemler, R., 1997. Beta-catenin is a target for the ubiquitin-proteasome pathway. Embo J. 16, 3797–3804.
Ahima, R.S., Flier, J.S., 2000. Adipose tissue as an endocrine organ. Trends Endocrinol. Metab. 11, 327–332.

Albalat, A., Liarte, C., MacKenzie, S., Tort, L., Planas, J.V., Navarro, I., 2005. Control of adipose tissue lipid metabolism by tumor necrosis factor-α in rainbow trout (*Oncorhynchus mykiss*). J. Endocrinol. 184, 527–534.

Boukouvala, E., Antonopoulou, E., Favre-Krey, L., Diez, A., Bautista, J.M., Leaver, M.J., Tocher, D.R., Krey, G., 2004. Molecular characterization of three peroxisome proliferator-activated receptors from the sea bass (*Dicentrarchus labrax*). Lipids 39, 1085–1092.

Bouraoui, L., Gutierrez, J., Navarro, I., 2008. Regulation of proliferation and differentiation of adipocyte precursor cells in rainbow trout (*Oncorhynchus mykiss*). J. Endocrinol. 198, 459–469.

Cawthorn, W.P., Sethi, J.K., 2008. TNF-α and adipocyte biology. FEBS Lett. 582, 117–131.

Cawthorn, W.P., Heyd, F., Hegyi, K., Sethi, J.K., 2007. Tumour necrosis factor-α inhibits adipogenesis via a β-catenin/TCF4 (TCF7L2)-dependent pathway. Cell Death Differ. 14, 1361–1373.

Corral, L.G., Haslett, P.A., Muller, G.W., Chen, R., Wong, L.M., Ocampo, C.J., Patterson, R.T., Stirling, D.I., Kaplan, G., 1999. Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-α. J. Immunol. 163, 380–386.

Cossu, G., Borello, U., 1999. Wnt signaling and the activation of myogenesis in mammals. Embo J. 18, 6867–6872.

Cruz-Garcia, L., Saera-Vila, A., Navarro, I., Calduch-Giner, J., Pérez-Sánchez, J., 2009. Targets for TNFα-induced lipolysis in gilthead sea bream (*Sparus aurata L.*) adipocytes isolated from lean and fat juvenile fish. J. Exp. Biol. 212, 2254–2260.

Dos Santos, J., Burkow, I.C., Jobling, M., 1993. Patterns of growth and lipid deposition in cod, *Gadus morhua L.*, fed natural prey and fish based feeds. Aquaculture 110, 173–189.

Du, Z.Y., Clouet, P., Zheng, W.H., Degrace, P., Tian, L.X., Liu, Y.J., 2006. Biochemical hepatic alterations and body lipid composition in the herbivorous grass carp (*Ctenopharyngodon idella*) fed high-fat diets. Br. J. Nutr. 95, 905–915.

Gao, W., Liu, Y.J., Tian, L.X., Mai, K.S., Liang, G.Y., Yang, H.J., Huai, M.Y., 2011. Protein sparing capability of dietary lipid in herbivorous and omnivorous freshwater finfish: a comparative case study on grass carp (*Ctenopharyngodon idella*) and tilapia (*Oreochromis niloticus* × *O. aureus*). Aquacult. Nutr. 17, 2–12.

Gebhardt, R., Hovhannisyan, A., 2010. Organ patterning in the adult stage: the role of Wnt/beta-catenin signaling in liver zonation and beyond. Dev. Dyn. 239, 45–55.

Hotamisligil, G., Shargill, N., Spiegelman, B.M., 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259, 87–91.

Huelsken, J., Behrens, J., 2002. The Wnt signalling pathway. J. Cell. Sci. 115, 3977–3978.

Langin, D., Arner, P., 2006. Importance of TNF alpha and neutral lipases in human adipose tissue lipolysis. Trends Endocrinol. Metab. 17, 314–320.

Lehwald, N., Tao, G.-Z., Jang, K.Y., Papandreou, I., Liu, B., Liu, B., Pysz, M.A., Willmann, J.K., Knoefel, W.T., Denko, N.C., Sylvester, K.G., 2012. Beta-catenin regulates hepatic mitochondrial function and energy balance in mice. Gastroenterology 143, 754–764.

Liu, J., Wang, H., Zuo, Y., Farmer, S.R., 2006. Functional interaction between peroxisome proliferator-activated receptor gamma and beta-catenin. Mol. Cell. Biol. 26, 5827–5837.

Liu, H., Fergusson, M.M., Wu, J.J., Rovira, I.I., Liu, J., Gavrilova, O., Lu, T., Bao, J., Han, D., Sack, M.N., Finkel, T., 2011. Wnt signaling regulates hepatic metabolism. Sci. Signal. 4 (158), ra6.

Liu, W., Singh, R., Choi, C.S., Lee, H.Y., Keramati, A.R., Samuel, V.T., Lifton, R.P., Shulman, G.I., Mani, A., 2012. Low density lipoprotein (LDL) receptor-related protein 6 (LRP6) regulates body fat and glucose homeostasis by modulating nutrient sensing pathways and mitochondrial energy expenditure. J. Biol. Chem. 287, 7213–7223.

Liu, D., Mai, K., Zhang, Y., Xu, W., Ai, Q., 2016. Wnt/β-catenin signaling participates in the regulation of lipogenesis in the liver of juvenile turbot (*Scophthalmus maximus* L.). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 191, 155–162.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using realtime quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408.

Loh, N.Y., Neville, M.J., Marinou, K., Hardcastle, S.A., Fielding, B.A., Duncan, E.L., McCarthy, M.I., Tobias, J.H., Gregson, C.L., Karpe, F., Christodoulides, C., 2015. LRP5 regulates human body fat distribution by modulating adipose progenitor biology in a dose-and depot-specific fashion. Cell Metab. 21, 262–272.

Luo, X., Li, H.X., Liu, R.X., Wu, Z.S., Yang, Y.J., Yang, G.S., 2009. Beta-catenin protein utilized by tumour necrosis factor-alpha in porcine preadipocytes to suppress differentiation. BMB Rep. 42, 338–343.

Moldes, M., Zuo, Y., Morrison, R.F., Silva, D., Park, B.H., Liu, J., Farmer, S.R., 2003. Peroxisome-proliferator- activated receptor gamma suppresses Wnt/beta-catenin signalling during adipogenesis. Biochem. J. 376, 607–613.

Mutoh, M., Niho, N., Wakabayashi, K., 2006. Concomitant suppression of hyperlipidemia and intestinal polyp formation by increasing lipoprotein lipase activity in Apc-deficient mice. Biol. Chem. 387, 381–385.

Nilsson-Ehle, P., Garfinkel, A.S., Schotz, M.C., 1980. Lipolytic enzymes and plasma lipoprotein metabolism. Annu. Rev. Biochem. 49, 667–693.

Oguma, K., Oshima, H., Aoki, M., Uchio, R., Naka, K., Nakamura, S., Hirao, A., Saya, H., Taketo, M.M., Oshima, M., 2008. Activated macrophages promote Wnt signalling through tumour necrosis factor-α in gastric tumour cells. EMBO J. 27, 1671–1681.

Richard, N., Kaushik, S., Larroquet, L., Panserat, S., Corraze, G., 2006. Replacing dietary fish oil by vegetable oils has little effect on lipogenesis, lipid transport and tissue lipid uptake in rainbow trout (*Oncorhynchus mykiss*). Br. J. Nutr. 96, 299–309.

Ridgeway, A.G., Petropoulos, H., Wilton, S., Skerjanc, I.S., 2000. Wnt signaling regulates the function of MyoD and myogenin. J. Biol. Chem. 275, 32398–32405.

Robinson, J.S., Mead, J.F., 1973. Lipid absorption and deposition in rainbow trout (*Salmo gairdnerii*). Can. J. Biochem. 51, 1050–1058.

Ross, S.E., Hemati, N., Longo, K.A., Bennett, C.N., Lucas, P.C., Erickson, R.L., MacDougald, O.A., 2000. Inhibition of adipogenesis by Wnt signaling. Science 289, 950–953.

Ruyter, B., Andersen, O., Dehli, A., Ostlund Farrants, A.K., Gjøen, T., Thomassen, M.S., 1997. Peroxisome proliferator activated receptors in Atlantic salmon (*Salmo salar*): effects on PPAR transcription and acyl-CoA oxidase activity in hepatocytes by peroxisome proliferators and fatty acids. Biochim. Biophys. Acta 1348, 331–338.

Saera-Vila, A., Calduch-Giner, J.A., Navarro, I., Pérez-Sánchez, J., 2007. Tumour necrosis factor (TNF) α as a regulator of fat tissue mass in the Mediterranean gilthead sea bream (*Sparus aurata L.*). Comp. Biochem. Physiol. Biochem. Mol. Biol. 146, 338–345.

Sánchez-Gurmaches, J., Cruz-Garcia, L., Gutiérrez, J., Navarro, I., 2012. MRNA expression of fatty acid transporters in rainbow trout: in vivo and in vitro regulation by insulin, fasting and inflammation and infection mediators. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 163, 177–188.

Sethi, J.K., Hotamisligil, G.S., 1999. The role of TNF alpha in adipocyte metabolism. Semin. Cell Dev. Biol. 10, 19–29.

Sheridan, M.A., 1988. Lipid dynamics in fish: aspects of absorption, transportation, deposition and mobilization. Comp. Biochem. Physiol. 90B, 679–690.

Song, K., Wang, S., Mani, M., Mani, A., 2014. Wnt signaling, de novo lipogenesis, adipogenesis and ectopic fat. Oncotarget 5, 11000–11003.

Tacon, A.G.J., 1996. Lipid nutritional pathology in farmed fish. Arch. Anim. Nutr. 49, 33–39.

Tefferi, A., Verstovsek, S., Barosi, G., Passamonti, F., Roboz, G.J., Gisslinger, H., Paquette, R.L., Cervantes, F., Rivera, C.E., Deeg, H.J., et al., 2009. Pomalidomide is active in the treatment of anemia associated with myelofibrosis. J. Clin. Oncol. 27, 4563–4569.

Thompson, M.D., Monga, S.P., 2007. WNT/beta-catenin signaling in liver health and disease. Hepatology 45, 1298–1305.

Vraskou, Y., Roher, N., Díaz, M., Antonescu, C.N., MacKenzie, S.A., Planas, J.V., 2011. Direct involvement of tumor necrosis factor-α in the regulation of glucose uptake in rainbow trout muscle cells. Am. J. Physiol. Regul. Integr. Comp. Physiol. 300, R716–R723.

Warne, J.P., 2003. Tumour necrosis factor: a key regulator of adipose tissue mass. J. Endocrinol. 177, 351–355.

Weisberg, S.P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R.L., Ferrante Jr., A.W., 2003. Obesity is associated with macrophage accumulation in adipose tissue. J. Clin. Invest. 112, 1796–1808.