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# Wnt/β-catenin signaling participates in the regulation of lipogenesis in the liver of juvenile turbot (*Scophthalmus maximus* L.)

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ABSTRACT

In this study, the mechanism that Wnt/β-catenin signaling inhibits lipogenesis was investigated in the liver of juvenile turbot (*Scophthalmus maximus* L.) by LiCl or XAV939 treatment. Wnt/β-catenin signaling was activated by LiCl treatment or inhibited by XAV939 treatment through regulating the expression of glycogen synthase kinase-3β (GSK-3β) and Wnt10b. In addition, the expression of lipoprotein lipase (LPL), fatty acid synthetase (FAS), peroxisome proliferator-activated receptor γ (PPARγ), and CCAAT/enhancer binding protein α (C/EBPα) was inhibited by LiCl treatment, but induced by XAV939 treatment. In the plasma of juvenile turbot, the level of nonesterified fatty acid (NEFA), glycerol, triglyceride (TG), total cholesterol (TC), and low density lipoprotein cholesterol (LDL-C) was decreased by LiCl treatment, which was related to the decrease of the activity of LPL and FAS. Thus the inhibitory effect of Wnt/β-catenin signaling on lipogenesis was associated with the expression of key enzymes and transcriptional factors. Wnt/β-catenin signaling may participate in inhibiting lipogenesis by inhibiting the expression of PPARγ and C/EBPα in the liver of juvenile turbot.



## 1. Introduction

Wnt subfamilies are an early development in the evolution of metazoan, which likely occurred about 650 Ma ago (Kusserow et al., 2005). At least 11 Wnt subfamilies have been found in the sea anemone (*Nematostella vectensis*) (Kusserow et al., 2005). In mammals, Wnt genes are classified into 12 subfamilies (Nusse, 2001; Prud'homme et al., 2002), in which Wnt10b specifically activates Wnt/β-catenin signaling (Barbolina et al., 2011; Wend et al., 2012). Wnt/β-catenin signaling is one of the negative signaling pathways on adipogenesis (Bennett et al., 2002; Prestwich and Macdougald, 2007), and β-catenin is involved in cell adhesion and transcriptional regulation (Ross et al., 2000). The expression level of β-catenin is associated with a destruction complex, mainly including glycogen synthase kinase-3β (GSK-3β), adenomatous polyposis coli (APC), and axin (Ross et al., 2000). In the absence of Wnt signals, β-catenin is phosphorylated by GSK-3β and degraded by ubiquitin (Aberle et al., 1997; Huelsken and Behrens, 2002). Nevertheless, Wnt ligands cause the inactivation of GSK-3β and cytosolic β-catenin accumulation, which will induce the target gene expression in the nucleus (Huelsken and Behrens, 2002). Moreover, the transcriptional factors CCAAT/enhancer binding proteins (C/EBP) and peroxisome proliferator-activated receptors (PPARs) play a significant role in the transition of pre-adipocytes into adipocytes (MacDougald and Lane, 1995). It has been found that Wnt/β-catenin signaling inhibits adipocyte differentiation by decreasing the expression of C/EBPα and PPARγ in 3T3-L1 preadipocytes (Ross et al., 2000; Huelsken and Behrens, 2002; Moldes et al., 2003).

Fish is a potential model for studying lipid metabolism due to its relatively high lipid accumulation in the liver and muscle (Robinson and Mead, 1973; Sheridan, 1988). Fatty acid synthetase (FAS) and lipoprotein lipase (LPL) play a key role in the regulation of hepatic lipid deposition (Nilsson-Ehle et al., 1980; Kerner and Hoppel, 2000; Richard et al., 2006). In addition, PPARγ participates in lipid homeostasis by orchestrating the gene transcription of the lipogenesis enzymes (Spiegelman and Flier, 2001). In fish species, PPARγ has been found in the Atlantic salmon (*Salmo salar*) (Ruyter et al., 1997), European sea bass (*Dicentrarchus labrax*) (Boukouvala et al., 2004), and gilthead sea bream (*Sparus aurata* L.) (Leaver et al., 2005). For the excessive accumulation of lipid that causes production losses, flesh quality alteration, and lipoid liver disease in fish, the regulation of lipid metabolism has become a notable point of interest in aquaculture research (Seierstad et al., 2005; 2009; Benedito-Palos et al., 2008; Saera-Vila et al., 2009). Since Wnt/β-catenin signaling affects adipogenesis, it is essential to investigate whether Wnt/β-catenin signaling is involved in the process of lipogenesis in fish species.

Juvenile turbot (*Scophthalmus maximus* L.) is an economically important flatfish species. In Wnt/β-catenin signaling, GSK-3β is an attractive target and lithium could activate Wnt/β-catenin signaling by inhibiting the activity of GSK-3β kinase (Terstappen et al., 2006). However, XAV939 displays nanomolar potency and antagonizes Wnt/β-catenin signaling by stabilizing Axin1 (Huang et al., 2009; Karlberg

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et al., 2010). The result of the previous study has shown that XAV939 blocks Wnt/β-catenin signaling in a mouse model (Yang et al., 2013). In this study, Wnt/β-catenin signaling was studied in the liver of juvenile turbot (*S. maximus* L.) by LiCl or XAV939 treatment. Furthermore, the mechanism that Wnt/β-catenin signaling regulates the expression of lipogenesis enzymes and transcriptional factors was investigated in the liver of juvenile turbot.

## 2. Materials and methods

### 2.1. Animals and experimental conditions

Juvenile turbot were obtained from a commercial farm (Qingdao, China) and transported to the indoor facilities in the experimental station of the Ocean University of China (Qingdao, China). At the beginning of the experiment, 135 fish (average body weight 50.32 ± 0.05 g) were randomly distributed into nine 400-L circular fiberglass flat-bottom tanks. In addition, another 10 fish were put in the tenth tank for tissue distribution analysis of Wnt signaling-related genes. Animals were fed once daily with a commercial diet (Qihao Biotech Co. Shandong, China) and acclimated for 15 days. All rearing tanks were maintained with a continuous aeration and a natural photoperiod. The water temperature was 18.0 ± 1 °C, the dissolved oxygen was approximately 7 mg/L, and the ammonia–nitrogen and nitrite were lower than 0.1 mg/L. All animal procedures were approved by the 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. Tissue distribution of Wnt signaling-related genes

After the acclimatization period and fasted for 24 h, five juvenile turbot (approximately 50.0 g) from the tenth tank were randomly sampled and immediately anesthetized. Then the tissue samples (stomach, intestine, spleen, heart, brain, kidney, liver, and muscle) were removed and frozen in liquid nitrogen to analyze the gene expression related to Wnt signaling.

### 2.3. Injection experiment

Juvenile turbot were injected intraperitoneally with 50 mg/mL LiCl (0.1 mg/g body weight), or 2 mg/mL XAV939 (4.0 μg/g body weight), or 0.9% sodium chloride (2 μL/g body weight) two times a week. LiCl (Sigma, America) or XAV939 (Medchem Express, America) was dissolved in 0.9% sodium chloride, respectively. Fish injected with sodium chloride were treated as the control. Three tanks were randomly used for the treatments of LiCl, XAV939, or the control group.

### 2.4. Sample collection

After two weeks, twelve fish from each tank were randomly sampled and anesthetized. In each tank, the livers from eight fish were removed, frozen in liquid nitrogen, and stored at −80 °C for subsequent analysis (the livers of five fish were pooled for biochemical analysis, and the livers of three fish were pooled for gene and protein expression analyses). Subsequently, the blood samples from another three fish were pooled with heparinized syringes, and plasmas were obtained after centrifugation for 10 min at 1400 × *g* (4 °C). Finally, the liver of the twelfth fish was fixed for 24 h in a solution of 4% paraformaldehyde dissolved in 0.1 mol/L sodium phosphate buffer (PBS, pH 7.4, 4 °C) for oil red O staining.

### 2.5. Biochemical analysis

Liver samples from each tank were homogenized in ice-cold 0.7% NaCl, and the supernatants were collected for biochemical analysis after centrifugation at 1400 × *g* for 10 min. For the nonesterified fatty acid (NEFA) that could bind with copper ion, NEFA content was assayed by determining the content of copper ion according to the analytical procedures in the nonesterified free fatty acid assay kit. Glycerol was measured based on the reaction of Trinder according to the analytical procedures in the glycerol assay kit. TG could be decomposed into glycerol and NEFA by LPL, and LPL activity was assayed based on the NEFA level according to the analytical procedures in the lipoprotein lipase kit. In addition, FAS catalyzes CoA and NADPH to generate long chain fatty acids, and FAS activity was assayed by determining the content of NADPH according to the procedures in the fatty acid synthase kit. All kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Finally, the protein concentration of liver supernatants was detected by the method of Coomassie Brilliant Blue G250 staining.

The level of total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) in plasma was measured according to the procedures of the total cholesterol assay kit, triglyceride assay kit, Low-density lipoprotein cholesterol assay kit, and high-density lipoprotein cholesterol assay kit (Nanjing Jiancheng Bioengineering Institute, China), respectively. The content of NEFA and glycerol in plasma was measured as the method described in the liver analysis.

### 2.6. Oil red O staining

The fixed liver tissues were washed three times with PBS (pH 7.4) and sectioned at a thickness of 20 μm in a cryostat (Leica CM1850, Germany), followed by mounting on the chromalum-gelatin-coated glass slides. Afterwards, the tissues were stained according to the procedures of oil red O staining kit (Nanjing Jiancheng Bioengineering Institute, China). Then the slides were re-dyed with hematoxylin and observed under a microscope.

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

Total RNA was extracted from the liver using TRIzol reagent (Invitrogen, USA) and electrophoresed on a 1.2% denaturing agarose gel to test the integrity. Then 3.0 μg total RNA was subjected to reverse transcription with EasyScript First-Strand cDNA Synthesis SuperMix Kit (TransGen, Beijing, China), and SYBR® Premix Ex Taq™ II was used to quantify the expression level of genes (TransGen, Beijing, China). The partial coding sequences for Wnt10b, GSK-3β, and β-catenin were cloned by PCR using degenerate primers deduced from the published sequences of other fish species (Table 1). The primer sequences for LPL, FAS, PPARγ, C/EBPα, Wnt10b, GSK-3β, β-catenin, and reference gene (β-actin) were designed following the published sequences and listed in Table 2. Real-time PCR was carried out in a quantitative thermal cycle (Mastercycler® ep realplex; Eppendorf, Germany). The real-time 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. The $2^{-\Delta\Delta CT}$ method was employed to analyze the differences of relative gene expression in each sample using β-actin as the internal reference gene (Livak and Schmittgen, 2001).

**Table 1**
Degenerate primers for gene clone of Wnt signaling in juvenile turbot.

| Target gene | Forward (5′–3′) | Reverse (5′–3′) |
|---|---|---|
| Wnt10b | GGSAGCTGCCAGTTYMAGA | CTCHTCRCASAKCACATA |
| GSK-3β | TYCGYCTGCGHTACTTCT | AWGGRTGGGCCTTRATCT |
| β-Catenin | AACCARGCCARCTGATTGCTGT | TGGAGGGWGTKCGTATGGAGG |

**Table 2**
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 |
|---|---|---|---|---|
| Wnt10b | TCCCAGAACAAGAACAATGG | ATCCCGCTCACAGAAGTCAG | 147 | KT372084 |
| 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 |

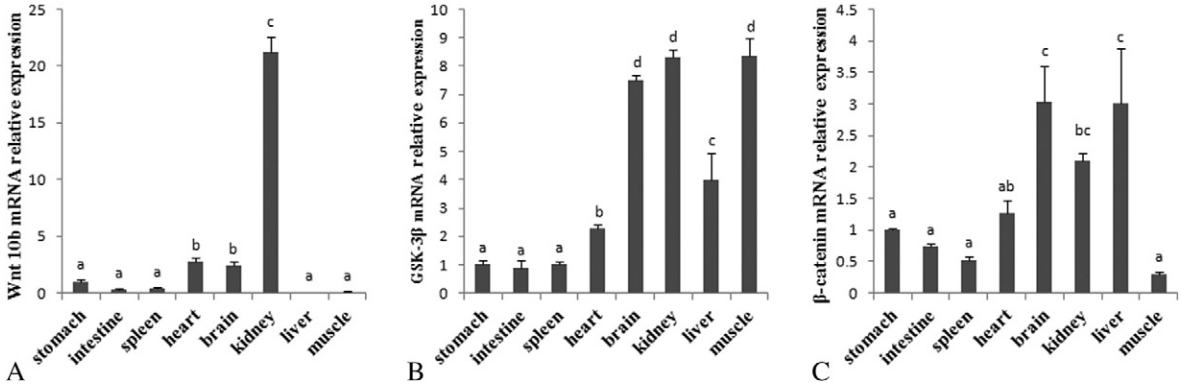


**Fig. 1.** The mRNA expression profile of Wnt signaling-related genes in the tissues of juvenile turbot. (A) Wnt10b; (B) GSK-3β; (C) β-catenin. Values are expressed as mean ± s.e.m. ($n = 5$ fish). Statistically significant differences are denoted by different letters.

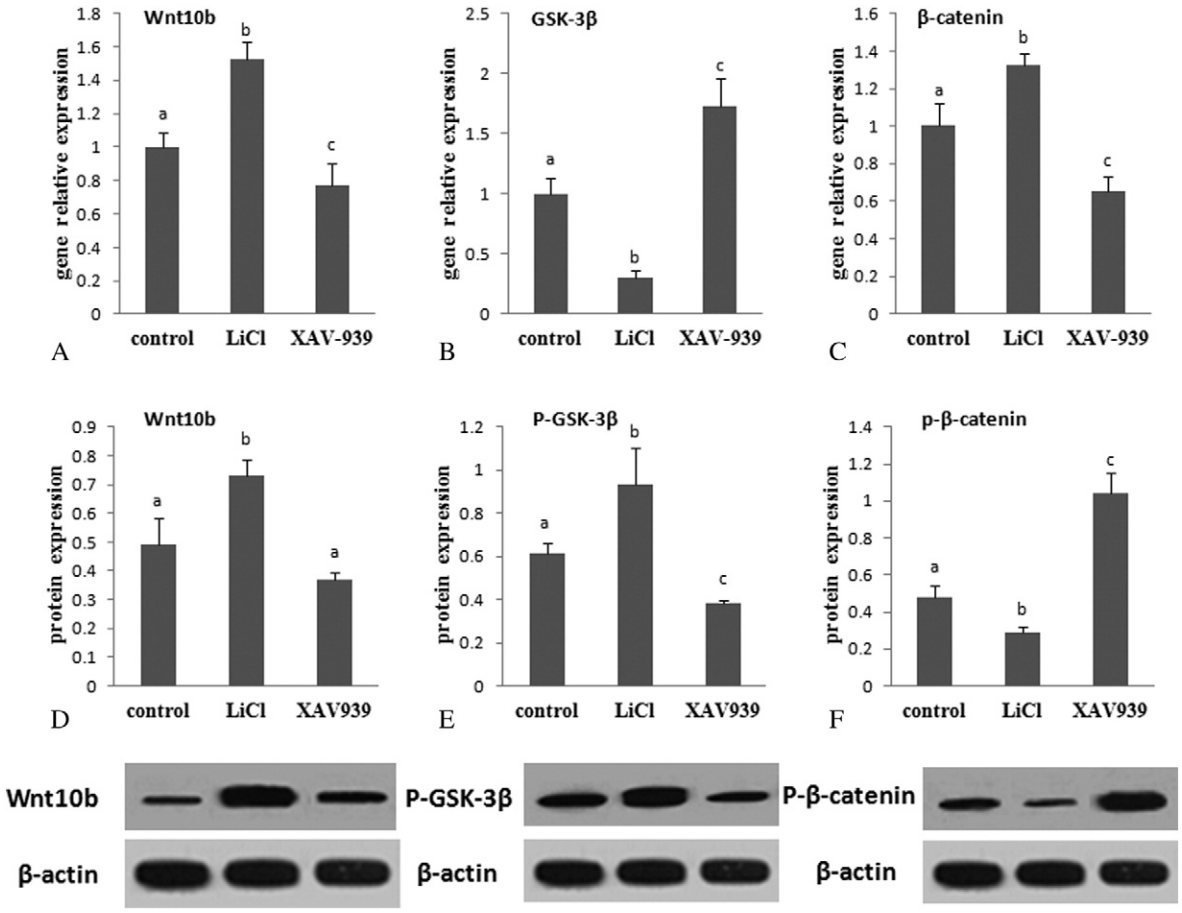


**Fig. 2.** Effect of LiCl and XAV939 on the mRNA and protein expression of Wnt signaling-related genes in the liver of juvenile turbot. (A) The mRNA expression of Wnt10b; (B) the mRNA expression of GSK-3β; (C) the mRNA expression of β-catenin; (D) the protein expression of Wnt10b; (E) the protein expression of p-GSK-3β (Ser9); (F) the protein expression of p-β-catenin (Ser33). Values are expressed as mean ± s.e.m. ($n = 3$ tanks). Statistically significant differences are denoted by different letters.

### 2.8. Western blot analysis

The frozen livers were homogenized on ice with a homogenizer (Iwaki, Chiba, Japan) 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 (Nanjing Jiancheng Bioengineering Institute, China). Homogenates were centrifuged at 1500 ×*g* for 15 min at 4 °C and supernatant fractions were then centrifuged at 20,000 ×*g* at 4 °C for 30 min. Protein samples were denatured by heating to 90 °C for 10 min in SDS-reducing buffer (0.5 M Tris–HCl pH 6.8, 10% glycerol, 10% SDS, 5% β-mercaptoethanol, 0.5% Brilliant Blue) and resolved by electrophoresis on 10% SDS-polyacrylamide gels. Nitrocellulose membranes were incubated with rabbit polyclonal antibody anti-p-β-catenin (Ser33) (sc-16743-R, Santa-Cruz, 1:500), goat polyclonal antibody anti-p-GSK-3β (Ser9) (sc-11757, Santa-Cruz, 1:500), rabbit polyclonal antibody anti-Wnt10b (sc-7432, 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-1615, Santa-Cruz, 1:1000), respectively. 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). The expression levels of proteins were evaluated using ImageJ software (NIH Image, Bethesda, MD, USA).

### 2.9. Statistical analysis

Data are presented as mean of three tank replicates and reported as mean ± standard error of means (s.e.m.). The normality and homogeneity of variances among groups were tested, and the results were subjected to one way ANOVA. Duncan's multiple range tests were used to determine the differences between the experimental groups if significance ($P < 0.05$) was identified. The statistical analyses were performed using SPSS 16.0 (SPSS Inc., 2005, USA).

## 3. Results

### 3.1. Tissue distribution of Wnt signaling-related genes

The highest mRNA expression level of Wnt10b was in the kidney followed by the heart and brain, but Wnt10b mRNA was expressed to a much lesser degree in the stomach, intestine, spleen, liver, and muscle of juvenile turbot (Fig. 1A). However, GSK-3β mRNA was most strongly expressed in the brain, kidney, and muscle, and weaker expression was observed in the heart and liver (Fig. 1B). The mRNA expression level of

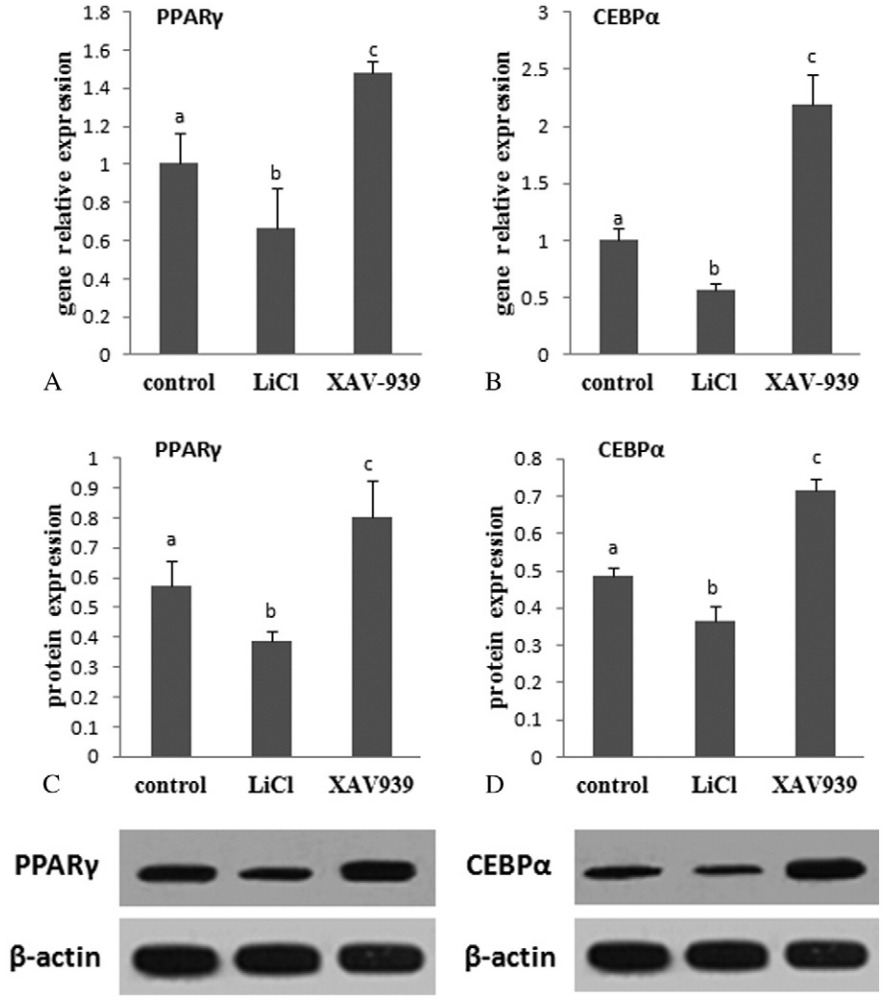


**Fig. 3.** Effect of LiCl and XAV939 on the mRNA and protein expression of peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer binding protein α (C/EBPα) in the liver of juvenile turbot. (A) The mRNA expression of PPARγ; (B) the mRNA expression of C/EBPα; (C) the protein expression of PPARγ; (D) the protein expression of C/EBPα. Values are expressed as mean ± s.e.m. ($n = 3$ tanks). Statistically significant differences are denoted by different letters.

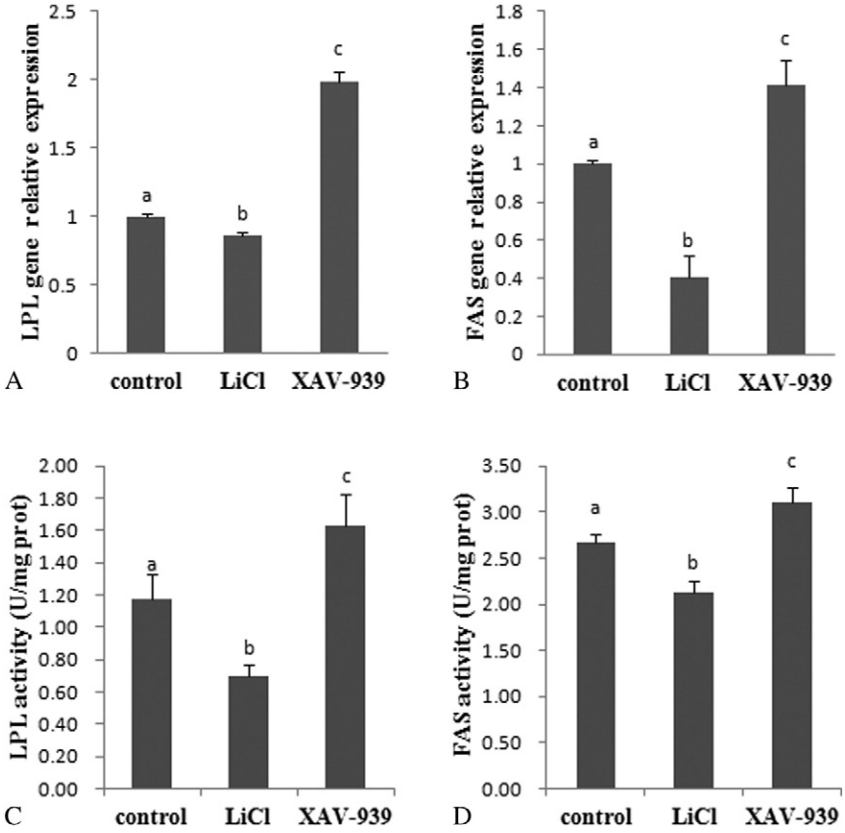


**Fig. 4.** Effect of LiCl and XAV939 on the mRNA expression and activity of lipoprotein lipase (LPL) and fatty acid synthetase (FAS) in the liver of juvenile turbot. (A) The mRNA expression of LPL; (B) the mRNA expression of FAS; (C) the activity of LPL; (D) the activity of FAS. Values are expressed as mean ± s.e.m. ($n = 3$ tanks). Statistically significant differences are denoted by different letters.

GSK-3β was lowest in the stomach, intestine, and spleen (Fig. 1B). Additionally, the mRNA of β-catenin was strongly expressed in the brain and liver followed by the kidney, while the lowest expression level was in the muscle (Fig. 1C).

### 3.2. Wnt signaling in the liver

The mRNA expression of Wnt10b and β-catenin was significantly induced by LiCl treatment (Fig. 2A, C), but significantly inhibited by XAV939 treatment (Fig. 2A, C). However, the mRNA expression of GSK-3β was significantly inhibited by LiCl treatment or significantly induced by XAV939 treatment (Fig. 2B).

In addition, the protein expression of Wnt10b and p-GSK-3β was significantly induced by LiCl treatment (Fig. 2D, E). Nevertheless, XAV939 treatment significantly inhibited the protein expression of p-GSK-3β (Fig. 2E). Moreover, the protein expression of p-β-catenin was significantly inhibited by LiCl treatment, but significantly induced by XAV939 treatment (Fig. 2F).

### 3.3. Effect of LiCl and XAV939 on PPARγ and C/EBPα expression

The mRNA expression of PPARγ and C/EBPα was significantly inhibited by LiCl treatment (Fig. 3A, B). However, XAV939 treatment significantly induced the mRNA expression of PPARγ and C/EBPα (Fig. 3A, B). In addition, the protein expression of PPARγ and C/EBPα was significantly inhibited by LiCl treatment, but significantly induced by XAV939 treatment (Fig. 3C, D).

### 3.4. Effect of LiCl and XAV939 on gene expression and activity of enzymes related to lipogenesis

After LiCl treatment, a clear reduction of LPL and FAS mRNA expression was observed (Fig. 4A, B). However, LPL and FAS mRNA expression

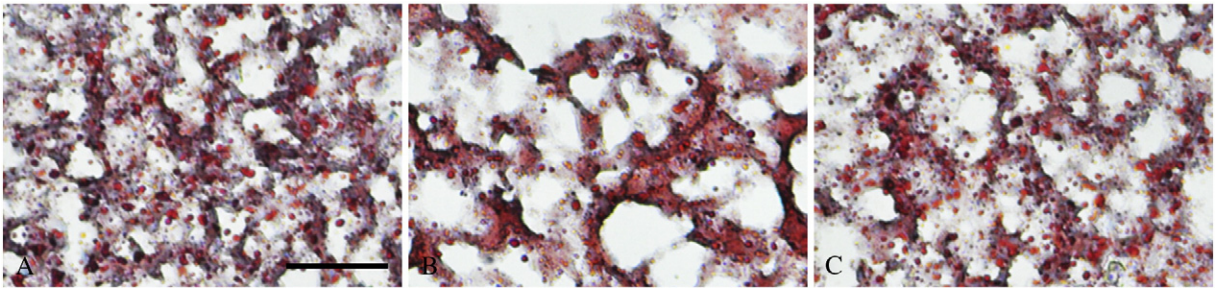


**Fig. 5.** Effect of LiCl and XAV939 on the lipid droplets in the liver of juvenile turbot. (A) Control; (B) LiCl treatment; (C) XAV939 treatment. Bar: 45 μm.

was significantly induced by XAV939 treatment (Fig. 4A, B). Moreover, the activity of LPL and FAS was significantly inhibited by LiCl treatment, but significantly induced by XAV939 treatment (Fig. 4C, D). Compared to the control, the number of lipid droplets was decreased by LiCl treatment, but XAV939 treatment increased the number of lipid droplets (Fig. 5).

### 3.5. Effect of LiCl and XAV939 on biochemical indexes in the liver and plasma

In the liver of juvenile turbot, the level of NEFA and glycerol was significantly decreased by LiCl treatment but significantly increased by XAV939 treatment (Fig. 6A, B). In plasma, the level of NEFA, glycerol,

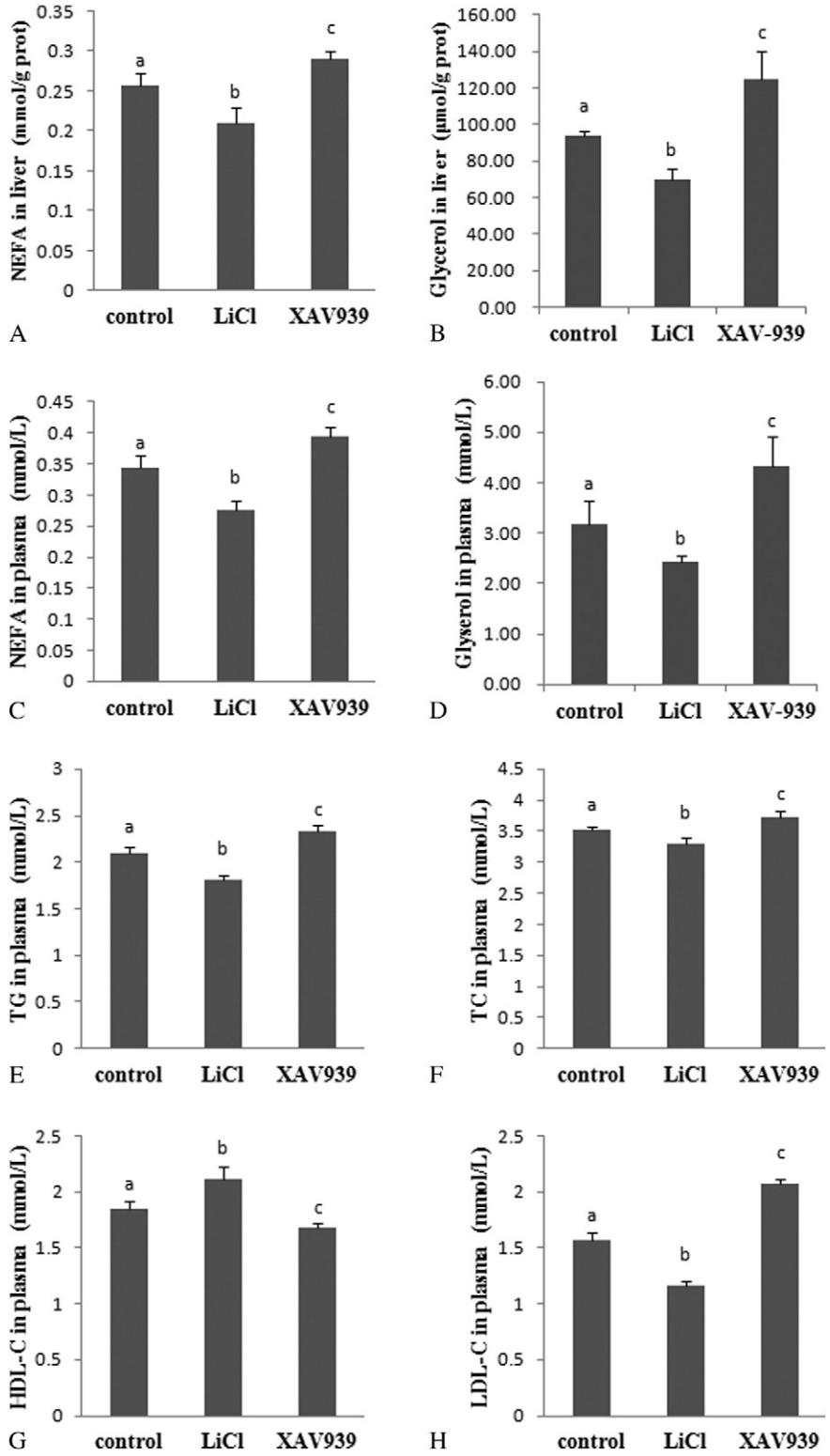


**Fig. 6.** Effect of LiCl and XAV939 on biochemical indexes in the liver and plasma of juvenile turbot. (A) Nonesterified fatty acid (NEFA) in the liver; (B) glycerol in the liver; (C) NEFA in plasma; (D) glycerol in plasma; (E) triglyceride (TG) in plasma; (F) total cholesterol (TC) in plasma; (G) high density lipoprotein cholesterol (HDL-C) in plasma; (H) low density lipoprotein cholesterol (LDL-C) in plasma. Values are expressed as mean ± s.e.m. ($n = 3$ tanks). Statistically significant differences are denoted by different letters.

TG, TC, and LDL-C was significantly decreased by LiCl treatment (Fig. 6C, D, E, F). However, a significant increase of NEFA, glycerol, TG, TC, and LDL-C was observed after XAV939 treatment (Fig. 6C, D, E, F). In addition, the level of HDL-C was significantly increased by LiCl treatment or decreased by XAV939 treatment (Fig. 6G, H).

## 4. Discussion

In this study, it was found that the mRNA expression of GSK-3β was inhibited and β-catenin expression was induced by LiCl treatment. The protein level of Wnt10b and p-GSK-3β was significantly induced by LiCl treatment. For GSK-3β activity that was negatively related to the level of p-GSK-3β, the activity of GSK-3β would be inhibited. Furthermore, the protein expression of p-β-catenin was inhibited by LiCl treatment, which would inhibit β-catenin degradation and lead to the cytoplasmic accumulation of β-catenin. However, XAV939 treatment inhibited Wnt/β-catenin signaling by increasing the expression of GSK-3β. The defining event in canonical Wnt signaling is the cytoplasmic accumulation of β-catenin (Aberle et al., 1997; Huelsken and Behrens, 2002), and GSK-3β plays a significant role in regulating β-catenin accumulation (Terstappen et al., 2006). Our results indicated that Wnt/β-catenin signaling was activated by LiCl treatment, but inhibited by XAV939 treatment in the liver of juvenile turbot (Fig. 7).

In fish, the liver is one of the main sites for lipid synthesis and storage (Robinson and Mead, 1973; Sheridan, 1988). The enzyme of FAS is one of the main lipogenic enzymes producing fatty acids (Richard et al., 2006). As a key enzyme in the lipid deposition, LPL hydrolyzes triacylglycerols in plasma lipoproteins, and supplies free fatty acids for storage or oxidation (Nilsson-Ehle et al., 1980). In addition, the fatty acids released by LPL will be taken up by adipocytes and accumulated in the lipid droplets. The gene of LPL has been analyzed in red sea bream (*Pagrus major*) and rainbow trout (Lindberg and Olivecrona, 2002; Oku et al., 2002). In this study, the expression and activity of LPL and FAS were decreased by LiCl treatment but increased by XAV939 treatment. It shows that Wnt/β-catenin signaling may inhibit the hepatic lipid deposition through inhibiting the activity of LPL and FAS (Fig. 7).

The development of adipose tissue is a continuous process, which includes the hypertrophy of existing adipocytes and the proliferation of new ones. The transcription factors act cooperatively to regulate the expression of genes associated with the adipocyte proliferation (Farmer, 2006). PPARγ and C/EBPα could induce the transcription of various proteins and enzymes involved in the differentiation of adipocytes (Shao and Lazar, 1997; Gregoire et al., 1998). In mammal studies, Wnt/β-catenin signaling blocks the induction of C/EBPα and PPARγ (MacDougald and Mandrup, 2002; Kawai et al., 2007). In this study, the expression of PPARγ and C/EBPα was inhibited by LiCl treatment but induced by XAV939 treatment. Thus Wnt/β-catenin signaling may inhibit the expression of PPARγ and C/EBPα, which further inhibits the expression of LPL and FAS (Fig. 7). However, the present study solely focuses on in vivo physiology, and other signaling pathways may participate in regulating lipogenesis. To investigate the specific effects of LiCl and XAV939 on lipogenesis, in vitro studies are needed to be done in the future.

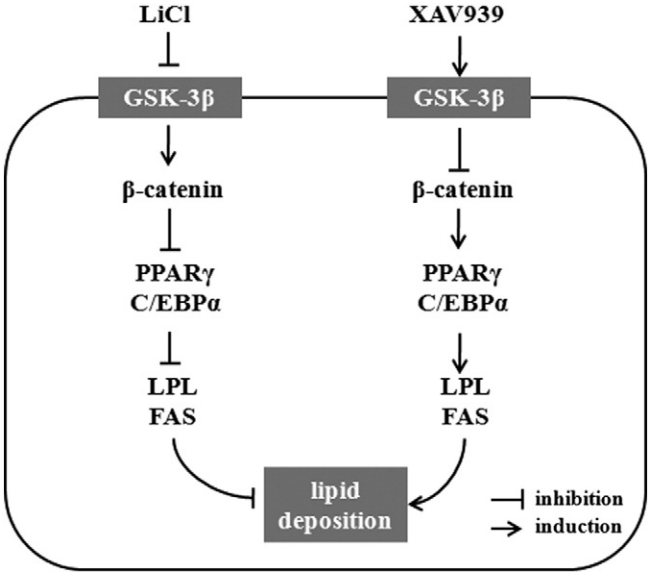


**Fig. 7.** Effect of LiCl and XAV939 on lipogenesis in the liver of juvenile turbot.

In the liver of juvenile turbot, the level of NEFA and glycerol was significantly decreased by LiCl treatment. In the plasma, the level of NEFA, glycerol, TG, TC, and LDL-C was decreased, and HDL-C was increased by LiCl treatment. However, XAV939 has an opposing effect on these biochemical indexes. Under normal physiological condition, there is a balance between lipid deposition and transportation to sustain lipid homeostasis in the liver. In addition, the endogenous and dietary lipids are delivered from the liver to the peripheral tissues for storage or use (Davis et al., 1979). HDL-C is involved in the process of reverse cholesterol transport, and LDL-C plays a key role in transporting cholesterol to the extrahepatic tissues. It has been found that the defective VLDL production and the subsequent reduced excretion of lipids or triglycerides induce hepatic lipidosis (Vance, 2008; Corbin and Zeisel, 2012). For LiCl treatment that significantly affects the biochemical indexes, Wnt/β-catenin signaling may regulate the levels of biochemical indexes through inhibiting the activity of LPL and FAS in the liver of juvenile turbot.

## 5. Conclusion

In summary, the mechanism that Wnt/β-catenin signaling inhibits lipogenesis was investigated in the liver of juvenile turbot (*S. maximus* L.) by LiCl or XAV939 treatment. Wnt/β-catenin signaling was activated by LiCl treatment or inhibited by XAV939 treatment through regulating the expression of GSK-3β and Wnt10b. In addition, the expression of LPL, FAS, PPARγ, and C/EBPα was inhibited by LiCl treatment, but induced by XAV939 treatment. In the liver and plasma, the level of biochemical indexes was regulated by LiCl or XAV939 treatment. Thus the inhibitory effect of Wnt/β-catenin signaling on lipogenesis was associated with the expression level of key enzymes and transcriptional factors. Wnt/β-catenin signaling may participate in inhibiting lipogenesis by inhibiting PPARγ and C/EBPα in the liver of juvenile turbot.

## Acknowledgments

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