



华东师范大学  
EAST CHINA NORMAL  
UNIVERSITY

Shanghai  
2016/7/27--2016/7/28

# The 2<sup>nd</sup> workshop on the study of lipid nutrition and metabolism in aquatic animals



# 1. Introduction

**21 participating units, nearly 70 participants;**

**17 invited lectures, 22 abstracts, 29 publications**



## 2. Invited lectures

- 1) **Sustainable feeds and n-3: impacts and solutions**——Douglas Tocher
- 2) **Recent advances in the study of regulatory mechanisms of LC-PUFA biosynthesis in rabbitfish**——Li Yuanyou
- 3) **Functional characterization and nutritional regulation of putative Elovl5 and Elovl4 elongases in large yellow croaker**——Ai Qinghui
- 4) **Is ARA an effective nutrient in depressing lipid accumulation and improving health status in grass carp**——Ji Hong
- 5) **Mechanisms and metabolic regulation of PPAR $\alpha$  activation in Nile tilapia**——Du Zhenyu
- 6) **The role of fatty acid desaturases on highly unsaturated fatty biosynthesis in loach**——Gao Jian

- 7) n-3 fatty acids impact on cardiometabolic diseases and obesity  
——Michel Nance
- 8) n-3 essential fatty acids in Nile tilapia: Bioconversion of dietary linolenic acid——Pan Qing
- 9) From marine environment to freshwater: in vivo synthesis of highly unsaturated fatty acids in *Litopenaeus vannamei*——Li Erchao
- 10) Effects of partial replacement of fish oil with rapeseed oil on growth, fatty acid profile and expression of immune related gene for two sizes of yellow croaker——Huang Xuxiong
- 11) The research of changed energy metabolism pattern of IGF-I overexpression Crucian Carp——Li Dongliang
- 12) The application of Maxi-mil in aquatic feed——Han Zejian

**13) Effects and mechanism of endoplasmic reticulum stress and the related signaling pathway on mineral-induced alteration in lipid metabolism in fish—Luo Zhi**

**14) n-3 rich diet as a nutritional strategy to counteract overactivation**

Focusing on the fatty acids metabolism (13);  
Focusing on the n-3 PUFA (9);  
Focusing on the n-3 PUFA biosynthesis (6)

**16) Cloning, tissue expression of the FABP gene, and effects of dietary phospholipid levels on FABP and vitellogenin mRNA expression in the female **swimming crab**—Zhou Qicun**

**17) The relationship among the host intestinal microbiota and environment—Zhang Meiling**

**Personal response:**

Ashamed

Tired

Gratified

Thanks for your attention!





## Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids

Volume 1861, Issue 9, Part A, September 2016, Pages 1036–1048



IF: 5.0

## Mechanisms and metabolic regulation of PPAR $\alpha$ activation in Nile tilapia (*Oreochromis niloticus*)

Li-Jun Ning<sup>1</sup>, An-Yuan He<sup>1</sup>, Jia-Min Li, Dong-Liang Lu, Jian-Gang Jiao, Ling-Yu Li, Dong-Liang Li, Mei-Ling Zhang, Li-Qiao Chen, Zhen-Yu Du  





# 1. Abstract

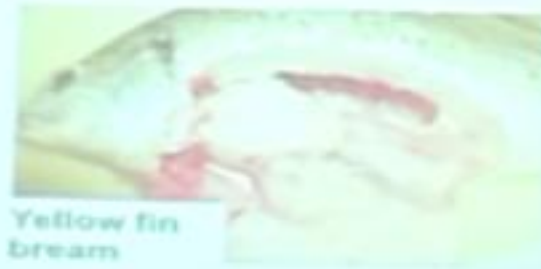
**Objective:** to illuminate the molecular mechanisms and metabolic regulation of PPAR $\alpha$  activation in fish

- Contents:**
- a. Gene cloning and tissue expression of mRNA of Nile tilapia (Nt) PPAR $\alpha$  .
  - b. Influence of the agonist (fenofibrate) and fasting on NtPPAR $\alpha$  expression in vitro and in vivo.
  - c. Investigating the metabolic regulatory effects of NtPPAR $\alpha$  (feed with fenofibrate or fasted).
  - d. Investigating the global regulatory effects of fenofibrate and fasting by hepatic transcriptomic study

# 1. Introduction

To spare the protein consumption, high-lipid diets are currently being used in aquaculture.

Severe lipid deposition in liver and viscera impairs fish health and food safety

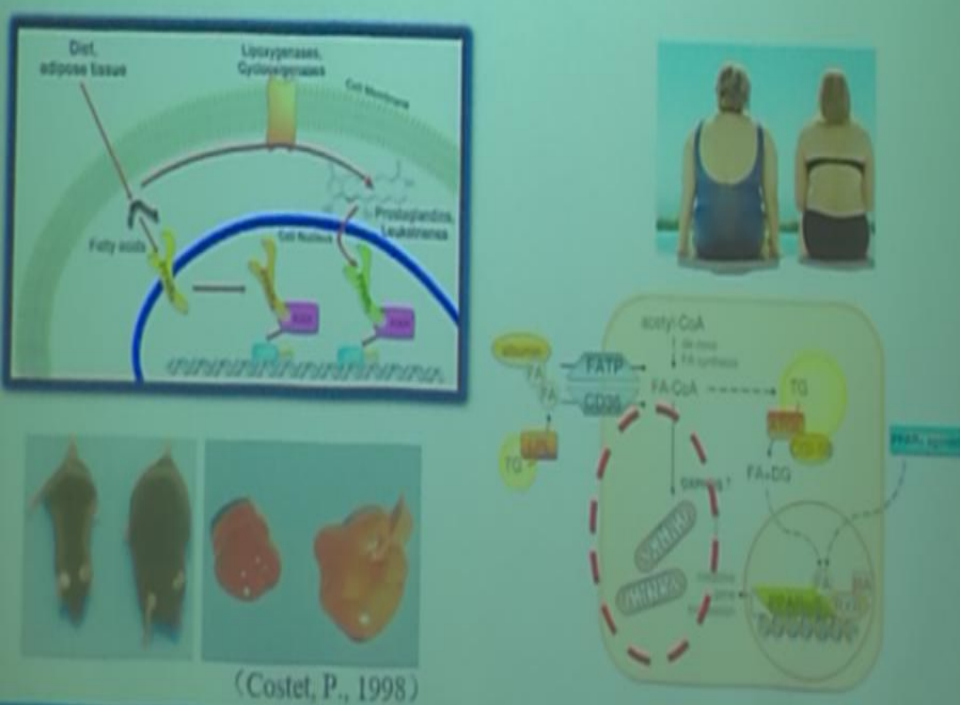


How to reduce the lipid deposition ?

# 1. Introduction

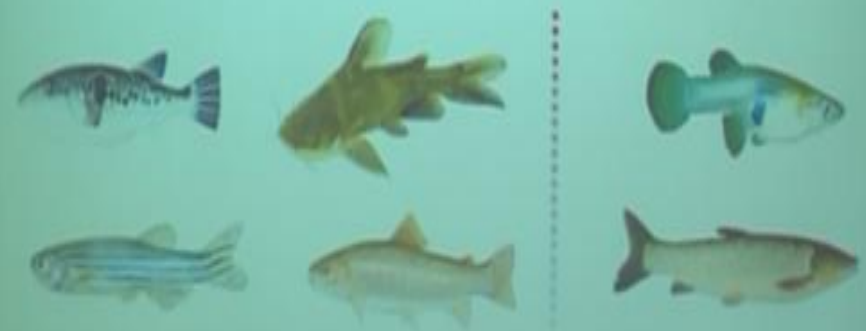
In mammals, PPAR $\alpha$  has been recognized as a master regulator of lipid metabolism, and a transcriptional factor activated by binding of ligands.

## PPAR $\alpha$ is a key regulator in lipid catabolism



## Fibrate-induced PPAR $\alpha$ activation in fish

PPAR $\alpha$  ligands, such as fibrates, have moderate lipid-lowering effects and induce PPAR $\alpha$  mRNA expression in torafugu, yellow catfish, rainbow trout gill-W1 cells, and zebrafish primary cells. However, bezafibrate and clofibrate acid have no effect on PPAR $\alpha$  expression in fathead minnow and grass carp.



(Du et al., 2004; Liu et al., 2005; Ibabe et al., 2005; Kondo et al., 2007; Du et al., 2008; Weston et al., 2009; Guo et al., 2015; Zheng et al., 2015; )

# 1. Introduction

PPAR $\alpha$  molecules have been cloned in many teleosts, but its activation mechanisms have not been well demonstrated.

PPAR $\alpha$  has been cloned in some fish species

Species	Highly expression	Literatures
牙鲆 Bastard halibut	Stomach, Liver, Intestine	(Cho et al, 2012)
草鱼 Grass carp	Liver	(He et al, 2012)
虹鳟 Rainbow trout	Adipose tissue, Muscle, Ovary	(贾成霞等, 2012)
军曹鱼 Cobia	Muscle, liver, Heart	(Tsai et al, 2008)
鲻鱼 Mullet	Liver	(Raigneard et al, 2006)
团头鲂 Black bream	Muscle, Muscle, Liver, Heart, Brain, Intestine	(Zhao et al, 2011)
黄颡鱼 Yellow catfish	Liver, Heart, Muscle	(Zhang et al, 2015a)
卵形鲳鲹 Pompano	Brain, Kidney, Intestine, Pleen	(方玲玲等, 2015)
尼罗罗非鱼 Nile Tilapia	Liver	(Ning et al, 2016)

The mechanisms of the PPAR $\alpha$  activation and the regulatory effects on lipid metabolism has not been thoroughly investigated in fish.



# 1. Introduction

Why choose the Nile tilapia as fish model for lipid metabolism?



**Nile tilapia** (*Oreochromis niloticus*) is an important aquaculture species worldwide and a good fish model for metabolic studies because of its rapid growth, high disease and stress resistance, and because its entire genome is available.

# 2. Materials and methods

## 2.1 feeding experiment and sampling

Two weeks acclimation with commercial diet

↓  
**HF diets, HF diets + fenofibrate, Fasting** (20 fish per tank)

↓  
Each fish was embedded with a RFID

6 fish of each group were euthanized and sampled at 3d, 1w and 4w

↓  
**Liver** (TG, glycogen, FA), **plasma** (TG,  $\beta$ -hydroxybutyrate, glucose, lactate, insulin and FFA)

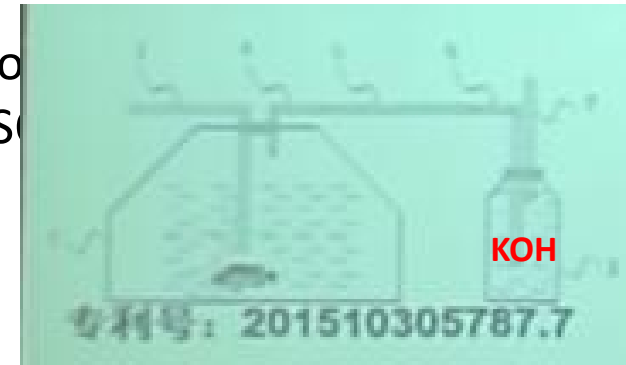


# 2. Contents

## 2.2. Gene cloning, identification and tissue expression of mRNA of NtPPAR $\alpha$

## 2.3. Catabolism rate of intraperitoneally injected [1-14C] palmitate

At the end of 4 week, four fishes from each group were sacrificed and received intraperitoneal injection Of [1-14C] palmitate (DMSO)



## 2.4. Mitochondrial and peroxisomal palmitate oxidation in liver and muscle homogenates

At the end of the feeding trial, pieces of liver and muscle were collected from each group, diluted, homogenized, measured.

# 2. Contents

**2.5. Culture of tilapia primary hepatocytes**

**2.6. Quantitative real time PCR and western blot analyses**

**2.7. Transcriptomic analysis**

**2.8. Histological study**



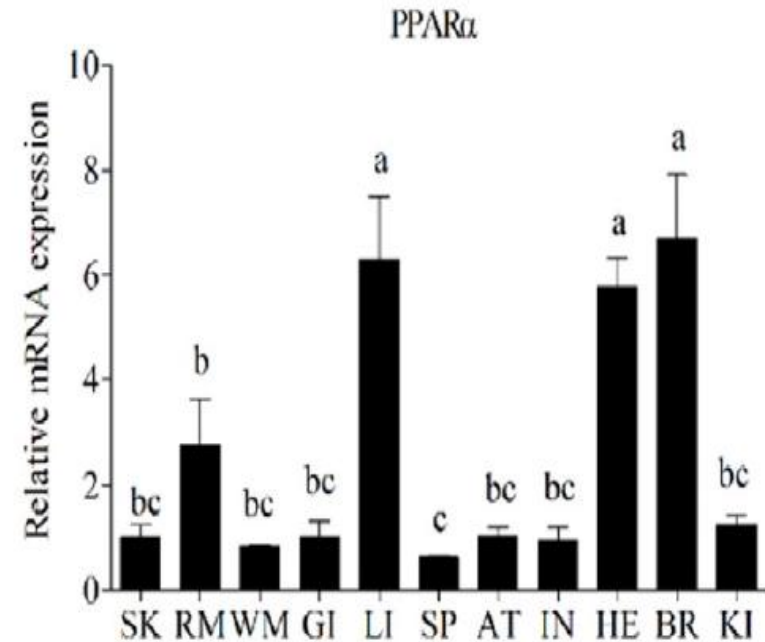
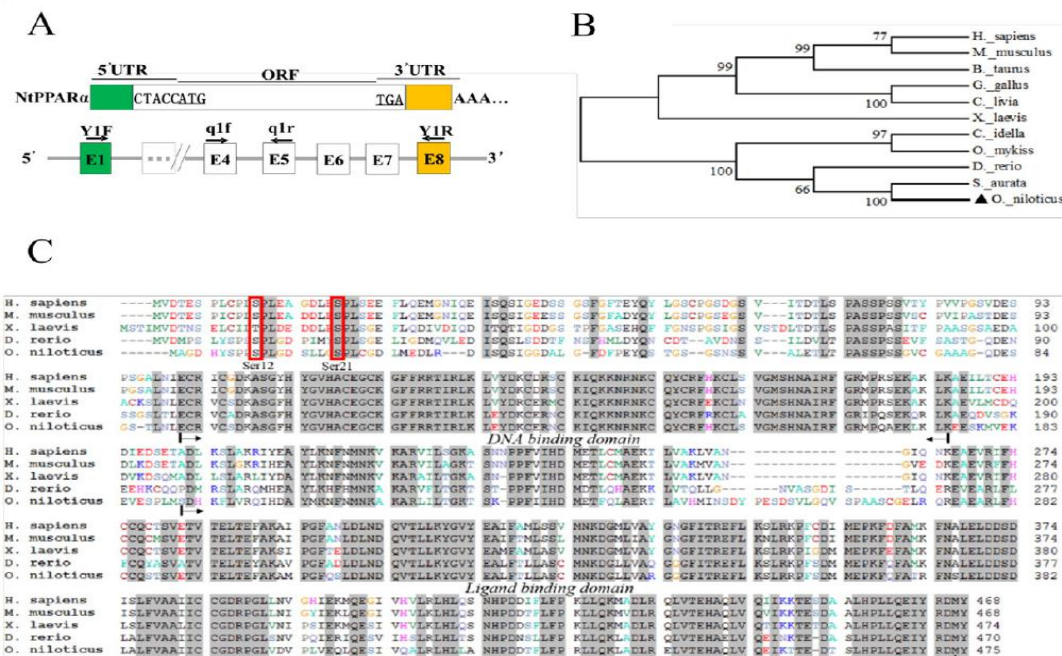


# 3. Results

## 3.1. NtPPAR $\alpha$ structure and features

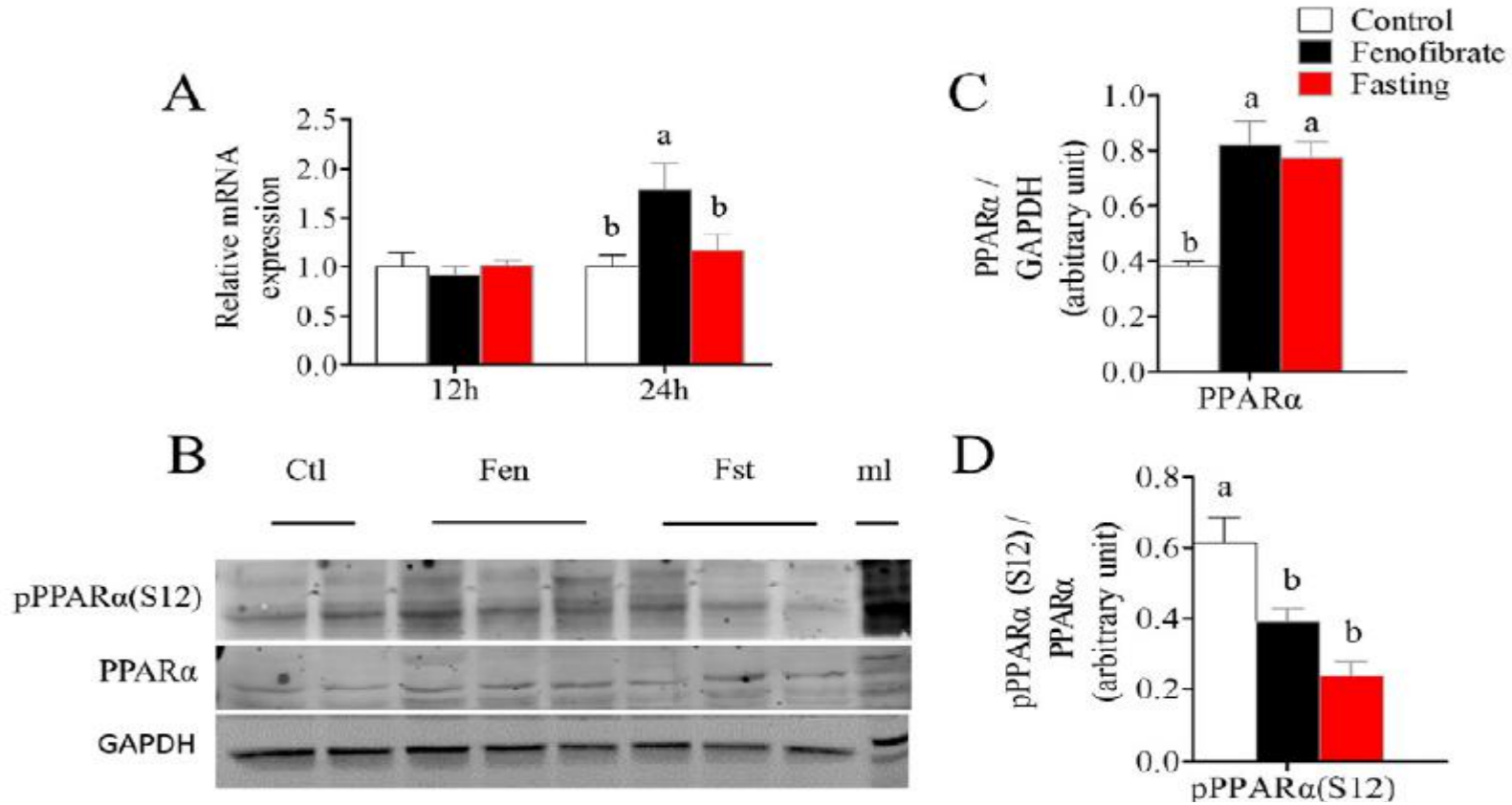
NtPPAR $\alpha$  has 475 deduced amino acid (AA), and share high identity to known PPAR $\alpha$  molecules in other teleosts

The highest expression level was detected in the brain, followed by the liver and heart



# 3. Results

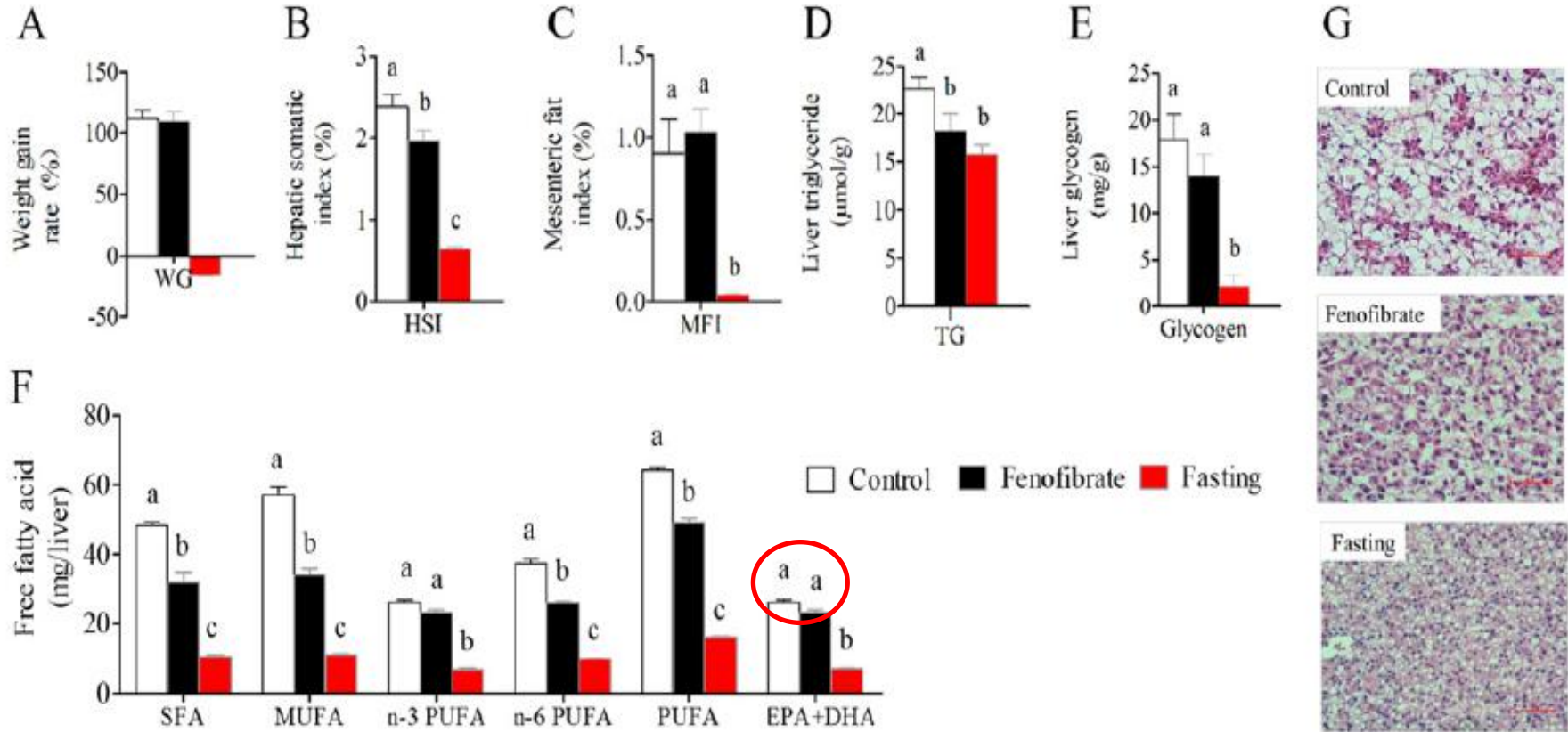
## 3.2. Activation of NtPPAR $\alpha$ by fenofibrate and fasting in primary hepatocytes of Nile tilapia



(A) The mRNA expression of NtPPAR $\alpha$  at 12 h and 24 h. (B) The protein express and phosphorylation of NtPPAR $\alpha$  using western blotting. (C–D) Statistical analysis of the western blotting results

# 3. Results

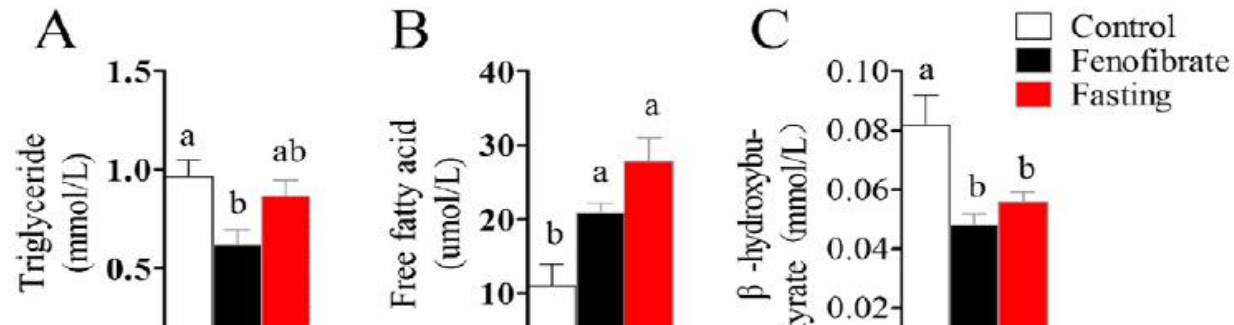
## 3.3. Effects of fenofibrate and fasting on body and serum composition



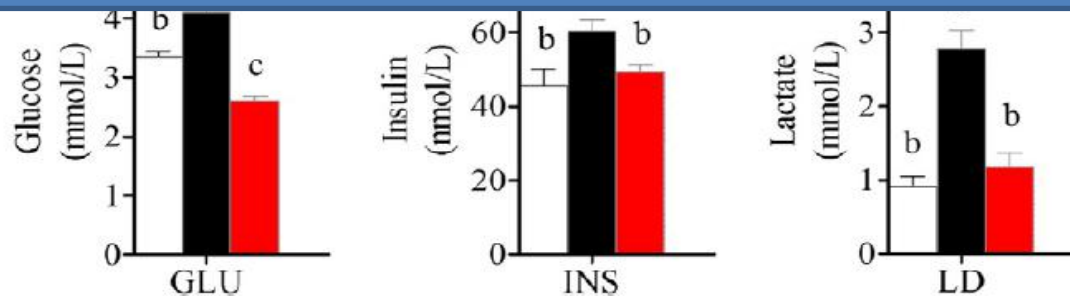
(A) Weight gain rate; (B) hepatic–somatic index (HSI); (C) mesenteric fat index; (D) hepatic triglyceride (TG) content; (E) hepatic glycogen content; (F) absolute amount of fatty acids in liver; (G) histological characteristics of liver.

# 3. Results

## 3.3. Effects of fenofibrate and fasting on body and serum composition



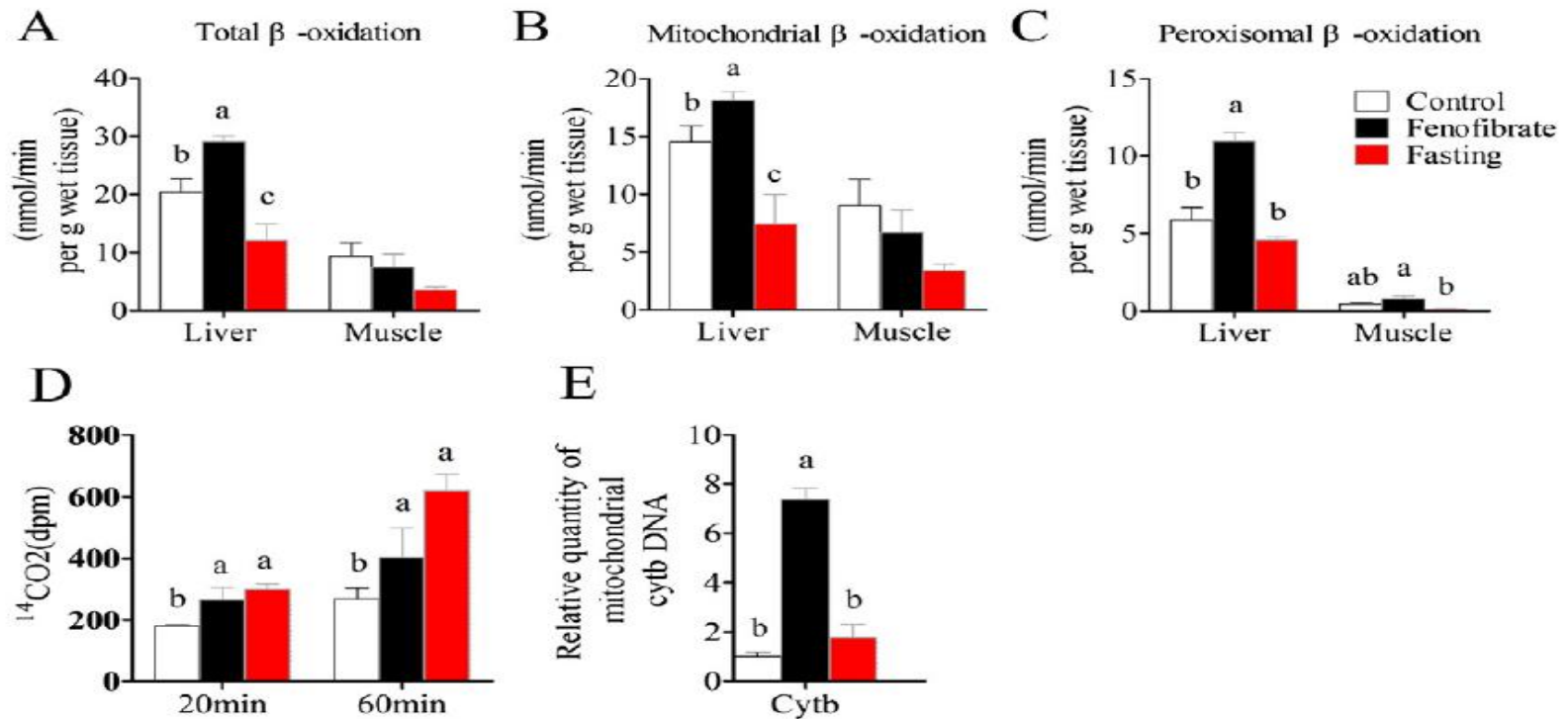
These results indicate that fenofibrate has hypolipidemic effects in tilapia and mainly targets the liver.



A) Triglyceride; B) free acids (FFAs); C)  $\beta$ -hydroxybutyrate; D) glucose; E) insulin; F) lactate.

# 3. Results

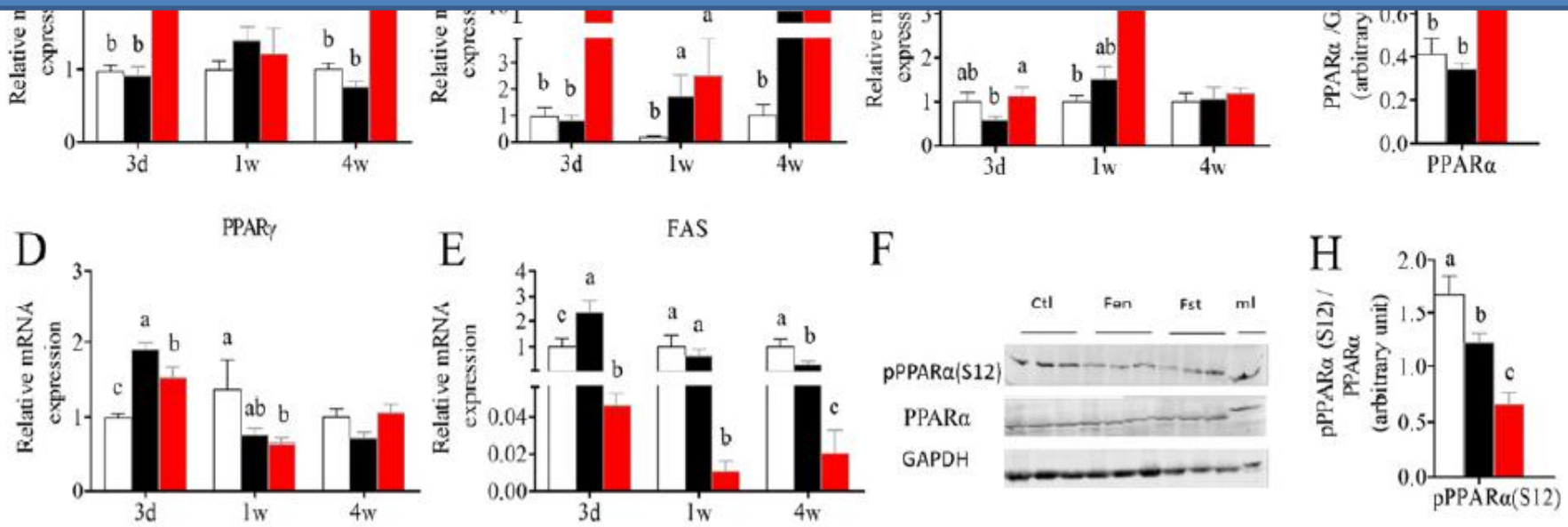
## 3.4. The hypolipidemic effects caused by fenofibrate are due to increased fatty acid $\beta$ -oxidation in tilapia



(A-C) The mitochondrial and peroxisomal  $\beta$ -oxidation of [1- $^{14}\text{C}$ ] palmitate in the homogenates of liver and muscle; (D) The conversion of intraperitoneally injected [1- $^{14}\text{C}$ ] palmitate into carbon dioxide; (E) The relative copy number of mtDNA cytochrome b

# 3. Results

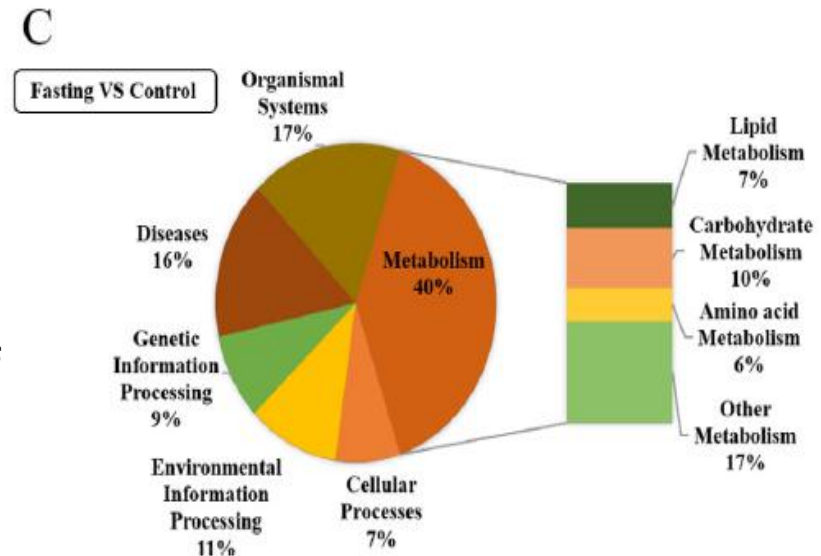
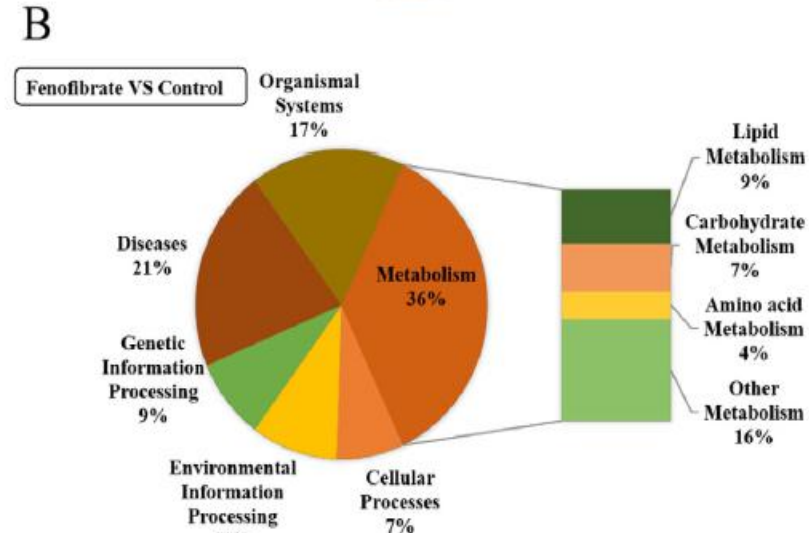
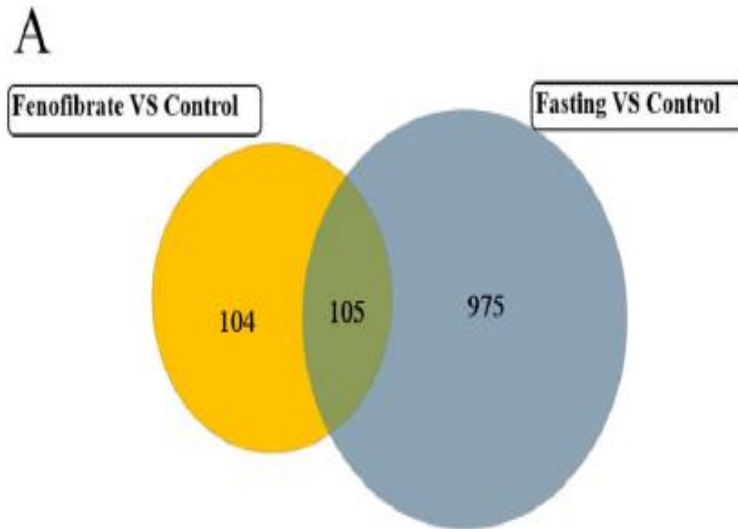
The results suggest that increased lipid breakdown and decreased adipogenesis are the main metabolic consequences after NtPPAR $\alpha$  is activated, the magnitude of NtPPAR $\alpha$  activation differed between the fenofibrate and fasting treatments, but dephosphorylation of NtPPAR $\alpha$  was likely the common activation mechanism in both treatments.



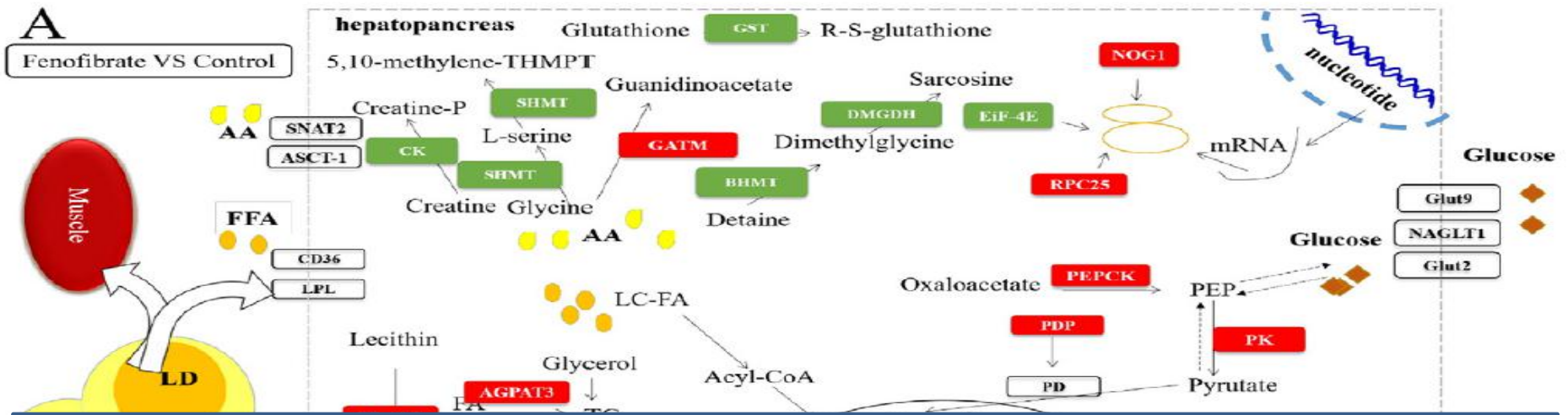
**A–C)** Lipid catabolism-related genes: PPAR $\alpha$ , CPT1a and ACO; **D–E)** Lipid anabolism related genes: PPAR $\gamma$  and FAS; **F)** western-blot of PPAR $\alpha$ , pPPAR $\alpha$  (S12) and GAPDH; **G)** relative WB quantification of PPAR $\alpha$ ; **H)** relative WB quantification of pPPAR $\alpha$  (S12).

# 3. Results

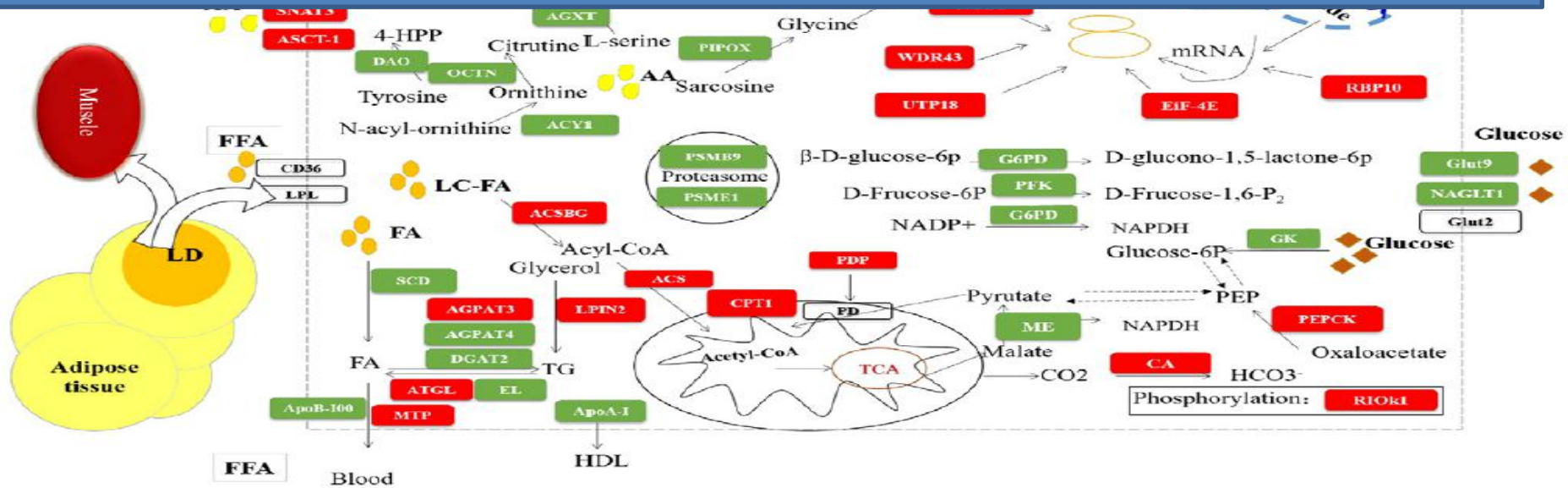
## 3.6. Global regulatory effects of fenofibrate and fasting on tilapia in a hepatic transcriptomic study



(A) Venn diagram representing mRNA transcripts differentially expressed (DEGs) in the liver from fasting and fenofibrate treatment compared to that of control. (B–C) Distribution by categories of differentially expressed genes and in the liver when compared fenofibrate and fasting treatment to that of control.



Specifically, significant changes were detected in genes of key enzymes for lipid hydrolysis (ATGL), adipogenesis (DGAT), and FA β-oxidation (ACS and CPT-I) in the transcriptomic measurements for the fasting group, whereas none of these genes changed significantly in the fenofibrate group.





# 4. Discussion

## 4.1. PPAR $\alpha$ activation and its mechanisms in different species

In mammals, two potential mechanisms for ligand-induced PPAR $\alpha$  activation have been proposed, **including increased receptor expression at the transcriptional or protein level** and **modifications of phosphorylation status (phosphorylation or dephosphorylation)**.

In the present study, in vitro and in vivo evidence for the first time, illustrate that NtPPAR $\alpha$  was activated by fenofibrate or endogenous ligands, such as fasting-induced FFAs, through **increased mRNA and protein expression** and **decreased phosphorylation**.



# 4. Discussion

## 4.2. Hypolipidemic effects and fatty acid $\beta$ -oxidation through PPAR $\alpha$ activation in different species

The hypolipidemic effects of PPAR $\alpha$  activation have been widely reported and one of the main mechanisms involves increasing mitochondrial and peroxisomal FA  $\beta$ -oxidation. However, these induction effects vary greatly among species.

Species	effects of fenofibrate	
	mitochondrial activity	peroxisomal activity
Rodents	increased	increased
Human	mildly induce	mildly induce
Monkey	mildly induce	mildly induce
Rainbow trout	increased	increased
Grass carp	increased	increased
Yellow catfish	increased	increased
Tilapia	increased	no

# 4. Discussion

## 4.3. NtPPAR $\alpha$ activation affects carbohydrate metabolism

Until now, there has been limited direct evidence linking pharmacological PPAR $\alpha$  activation and glucose homeostasis. Our results show higher serum levels of glucose, insulin, and lactate in the fenofibrate group than those in the fasting group, indicating that glucose utilization decreased in response to fenofibrate. Those studies suggest that utilization of lipid and carbohydrate is balanced in fish.



# 5. Conclusions

## Conclusions

- PPAR $\alpha$  was activated in response to fenofibrate and fasting in Nile tilapia;
- NtPPAR $\alpha$  activation mainly targets the liver and is relatively moderate;
- Dephosphorylation is the basal NtPPAR $\alpha$  activation mechanism;
- NtPPAR $\alpha$  activation increased activity and the number of hepatic mitochondria.





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Thanks for your attention!

