Dietary L-carnitine supplementation increases lipid deposition in the liver and muscle of yellow catfish (*Pelteobagrus fulvidraco*) through changes in lipid metabolism

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Abstract
Carnitine has been reported to improve growth performance and reduce body lipid content in fish. Thus, we hypothesised that carnitine supplementation can improve growth performance and reduce lipid content in the liver and muscle of yellow catfish (*Pelteobagrus fulvidraco*), a commonly cultured freshwater fish in inland China, and tested this hypothesis in the present study. Diets containing L-carnitine at three different concentrations of 47 mg/kg (control, without extra carnitine addition), 331 mg/kg (low carnitine) and 3495 mg/kg (high carnitine) were fed to yellow catfish for 8 weeks. The low-carnitine diet significantly improved weight gain (WG) and reduced the feed conversion ratio (FCR). In contrast, the high-carnitine diet did not affect WG and FCR. Compared with the control diet, the low-carnitine and high-carnitine diets increased lipid and carnitine contents in the liver and muscle. The increased lipid content in the liver could be attributed to the up-regulation of the mRNA levels of SREBP, PPAR, fatty acid synthase (FAS) and ACCa and the increased activities of lipogenic enzymes (such as FAS, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and malic enzyme) and to the down-regulation of the mRNA levels of the lipolytic gene CPT1A. The increased lipid content in muscle could be attributed to the down-regulation of the mRNA levels of the lipolytic genes CPT1A and ATGL and the increased activity of lipoprotein lipase. In conclusion, in contrast to our hypothesis, dietary carnitine supplementation increased body lipid content in yellow catfish.

Key words: *Pelteobagrus fulvidraco*: Dietary L-carnitine: Growth performance: Lipid deposition: Lipid metabolism

Abstract

Carnitine has been reported to improve growth performance and reduce body lipid content in fish. Thus, we hypothesised that carnitine supplementation can improve growth performance and reduce lipid content in the liver and muscle of yellow catfish (*Pelteobagrus fulvidraco*), a commonly cultured freshwater fish in inland China, and tested this hypothesis in the present study. Diets containing L-carnitine at three different concentrations of 47 mg/kg (control, without extra carnitine addition), 331 mg/kg (low carnitine) and 3495 mg/kg (high carnitine) were fed to yellow catfish for 8 weeks. The low-carnitine diet significantly improved weight gain (WG) and reduced the feed conversion ratio (FCR). In contrast, the high-carnitine diet did not affect WG and FCR. Compared with the control diet, the low-carnitine and high-carnitine diets increased lipid and carnitine contents in the liver and muscle. The increased lipid content in the liver could be attributed to the up-regulation of the mRNA levels of SREBP, PPAR, fatty acid synthase (FAS) and ACCa and the increased activities of lipogenic enzymes (such as FAS, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and malic enzyme) and to the down-regulation of the mRNA levels of the lipolytic gene CPT1A. The increased lipid content in muscle could be attributed to the down-regulation of the mRNA levels of the lipolytic genes CPT1A and ATGL and the increased activity of lipoprotein lipase. In conclusion, in contrast to our hypothesis, dietary carnitine supplementation increased body lipid content in yellow catfish.

Key words: *Pelteobagrus fulvidraco*: Dietary L-carnitine: Growth performance: Lipid deposition: Lipid metabolism

**L-Carnitine** (β-OH-γ-N-(trimethylammonio)butyrate) is a water-soluble quaternary amine that occurs naturally in micro-organisms, plants and animals(1). It plays an important role in lipid metabolism and energy production by chaperoning activated fatty acids (acyl coenzyme A) into the mitochondrial matrix and transporting intermediate compounds out of the matrix to prevent their accumulation(2). In all animals including fish, L-carnitine can be biosynthesised from lysine(3). However, in fast-growing juvenile animals, endogenous carnitine synthesis is insufficient to meet the energy requirement(4). Thus, dietary L-carnitine supplementation is essential. During the last two decades, many studies have been conducted to determine the effects of dietary carnitine concentrations on growth performance and lipid deposition in fish. In several studies, L-carnitine has been found to be beneficial for growth performance(5–9) and to reduce body lipid content(5,10,11).

In general, fat accumulation results from the balance between dietary absorbed fat, *de novo* synthesis of fatty acids (lipogenesis) and fat catabolism via β-oxidation (lipolysis), and many key enzymes and transcription factors are involved in this process. These enzymes include lipogenic enzymes (such as 6-phosphogluconate dehydrogenase (6PGD), glucose-6-phosphate dehydrogenase (G6PD), malic enzyme (ME), isocitrate dehydrogenase (ICDH), fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC)) and lipolytic enzymes (such as carnitine palmitoyltransferase 1 (CPT1), hormone-sensitive lipase (HSL) and adipose TAG lipase (ATGL))(12). Lipoprotein lipase (LPL) hydrolyses TAG present in plasma lipoproteins and supplies NEFA for storage in the...
adipose tissue, or for oxidation in other tissues, and plays a pivotal role in the regulation of lipid content in fish\(^{12}\). On the other hand, several transcription factors, such as PPAR\(\alpha\), PPAR\(\gamma\) and sterol regulatory element-binding protein-1 (SREBP-1), play an intermediary role in lipid homeostasis, by orchestrating the gene transcription of enzymes involved in this pathway\(^{13}\). Although many studies have investigated the effects of dietary carnitine concentrations on lipid deposition in fish, the underlying molecular processes involved in the alteration of lipid deposition as a response to dietary L-carnitine are not yet known.

Yellow catfish (*Pelteobagrus fulvidraco*), an omnivorous freshwater fish, is considered to be a good candidate for freshwater culture in China due to its delicious meat and high market value. However, excessive lipid deposition, which may affect the quality of harvest, in yellow catfish has been reported to be a problem in some fish farms. Recently, we cloned and characterised the complementary DNA (cDNA) sequences and structures of genes involved in lipid metabolism in this fish species\(^{14}\), facilitating further studies to be carried out on the regulation of lipid metabolism at the molecular level. We hypothesised that dietary carnitine supplementation can improve growth performance and reduce lipid deposition in yellow catfish and tested this hypothesis in the present study. The effects of dietary L-carnitine were evaluated by determining growth performance and lipid deposition and by investigating the activities of enzymes involved in lipid metabolism. The mRNA levels of twelve pivotal genes involved in lipid metabolism, including *CPT1A*, *LPL*, *ACCa*, *ACCh*, *FAS*, *G6PD*, *6PGD*, *ATGL*, *HSL*, *PPAR\(\alpha\)*, *PPAR\(\gamma\)* and SREBP-1, were determined by quantitative real-time PCR using a set of gene-specific primers. The activities of six enzymes involved in lipogenesis, including LPL, FAS, G6PD, 6PGD, ME and ICDH, were also determined. The liver and muscle were sampled to determine whether dietary L-carnitine supplementation affects lipid metabolism in a tissue-specific manner. To our knowledge, the present study is the first to determine the effects of dietary L-carnitine concentrations on lipid deposition and metabolism at both the enzymatic and molecular levels, and in two tissues (the liver and muscle) and gain new insights into carnitine nutrition in fish.

**Materials and methods**

**Diet preparation**

A commercial feed was used as a basal diet. The basal diet without extra carnitine supplementation was used as the control diet; two other diets were supplemented with pure L-carnitine inner salt (Sigma–Aldrich) at concentrations of 500 and 3500 mg/kg, respectively (Table 1). The highest concentration of carnitine used in the present study was similar to that used in a study on African catfish\(^{15}\). Carnitine concentrations in the three experimental diets used in the present study were 47 mg/kg (control), 331 mg/kg (low) and 3495 mg/kg (high) diet, respectively. The feed was produced according to the method used in our recent study\(^{16}\).

All pellets were dried at 40°C using an air blower until the moisture was reduced to <10%. The dried pellets were placed in plastic bags and stored at −20°C until fed to the fish.

**Animals and experimental procedure**

Yellow catfish larvae (mean initial weight: 80 mg) were obtained from a local fish farm (Wuhan, China) and transferred into 300-litre circular fibreglass tanks for 3-week acclimatisation. During acclimatisation, fish were fed a live diet (rotifer) mixed with the control diet for 4 weeks three times daily. The amount of rotifer was greater than that of the control diet before acclimatisation, but the amount of the diet was progressively increased until the fish readily consumed the diet. At the start of the experiment, thirty-five uniformly sized fish (mean initial weight: 0.15 ± 0.02 g) in good condition were stocked in each of the fibreglass tanks. Each diet was assigned to three tanks in a completely randomised manner, resulting in nine tanks in the experiment. The diets were fed to the fish by hand to apparent satiation twice daily (09.00 and 16.00 hours). Care was taken to ensure that no uneaten feed remained in the tanks during feeding. The amount of feed consumed by the fish in each tank was recorded daily. Faecal matter was also quickly removed during the experiment. Water in each tank was replenished 100% daily. The tanks were aerated to maintain the dissolved oxygen levels at saturation. Mortality was monitored daily. The experiment was carried out for 8 weeks.

The experiment was conducted at ambient temperature under natural photoperiod conditions (approximately 14 light–10 h dark). Water quality parameters were monitored once daily in the morning. Water temperature, pH, and dissolved oxygen, ammonia and nitrite levels were 30.5 ± 2.5°C, 7.4 ± 0.1, 6.0 (SE ± 0.2) mg/l, 0.17 (SE ± 0.08) mg/l and 0.04 (SE ± 0.01) mg/l, respectively.

**Sampling and sample analysis**

At the end of the experiment, 24 h after the last feeding, fish were killed (tricaine methanesulfonate at a dose of 10 mg/l). All fish were counted and weighed in batches to determine the survival rate and weight gain. Then, fish were dissected on ice to obtain livers and muscle. The livers were also weighed to calculate the hepatosomatic index. Crude lipid content in the liver and muscle was determined by diethyl ether extraction, and it is expressed as g crude lipid/100 g.
For histochemical observation, frozen livers were cut on a cryostat microtome. Sections measuring 9 μm in thickness were fixed in 4% formaldehyde for 10 min and stained with Oil Red O and then prepared for light microscopy, according to the method of Lillie & Fullmer(16).

For the determination of total carnitine content, total carnitine was isolated according to the method of Alhomida et al. With slight modifications proposed by Zheng et al. First, samples (0.5 g) were homogenised in 1 ml of ice-cold 1.2 M-HClO₄ using a PowGen polytron (Fisher Scientific). After centrifugation at 8000 g for 10 min, the pellet was washed twice by suspending in 0.5 ml of 0.6 M-HClO₄ and centrifuged again. Then, 0.3 ml of tissue homogenate were mixed with 0.2 ml of 5 M-KOH and heated at 80°C for 1 h. After cooling, the extract was adjusted to a pH of 7.0 with ice-cold 1.2 M-HClO₄. Carnitine content was measured using the enzymatic radioisotope method(19).

Enzyme activity determination

For the determination of LPL activity, samples were homogenised in 1 ml of 1.2 M-HClO₄ and centrifuged at 8000 g for 15 min at 4°C. The supernatant was used for the determination of LPL activity. The activity of LPL was measured using labelled triolein-3H as a substrate, according to the modified methods of Ballart et al. The changes in absorbance at 340 nm were monitored at intervals of 15 s for 3 min. The activities of 6PGD and G6PD were determined using the method of Barroso et al. The activity of ME using the method of Wise & Ball, that of ICDH according to the method of Bernt & Bergmeyer, and that of FAS according to the method of Chakrabarty & Leveille. One unit of enzyme activity (IU), defined as the amount of enzyme that converts 1 μmol of substrate to product/min at 30°C, is expressed as units per mg of soluble protein. The soluble protein concentration of the homogenates was determined by the method of Bradford using bovine serum albumin as a standard.

Quantitative RT-PCR

The extraction of total RNA and synthesis of first-strand cDNA were carried out according to the methods used in our recent study with slight modifications. The cDNA synthesis reaction mixtures were diluted to 200 μl in water. Quantitative PCR (20 μl) was carried out in ninety-six-well plates in a Bio-Rad iCycler iQ™ real-time PCR system (MyiQ, iCycler) with GoTaq® qPCR Master (Promega), containing 10 μl of GoTag® qPCR Master Mix, 2 μl of cDNA and 0.2 μmol/l of each primer. The primer sequences of each gene used in this analysis are given in Table 2. The thermal programme included 1 min at 95°C and forty-five cycles at 95°C for 5 s, 57°C for 10 s, and 72°C for 30 s. All reactions were carried out in duplicate, and each reaction mixture was checked to ensure that it contained a single product of the correct size by agarose gel electrophoresis. A no-template control test and a dissociation curve analysis were carried out to ensure

### Table 2. Primers used in real-time PCR analysis

<table>
<thead>
<tr>
<th>Genes</th>
<th>Forward primer (5′–3′)</th>
<th>Reverse primer (5′–3′)</th>
<th>Size (bp)</th>
<th>Accession no.</th>
</tr>
</thead>
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<td>ATTTGAAGAAGACCCAGAGTAGTGT</td>
<td>CCCCCCTTATGAGGGAGAAGACA</td>
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<td>PPARα</td>
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<td>JX929740</td>
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<tr>
<td>PPARγ</td>
<td>ACCGCCCTCTCCCTATCC</td>
<td>TGGACAGACTCAGTCAGATG</td>
<td>360</td>
<td>JX929741</td>
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<td>SREBP-1</td>
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<td>188</td>
<td>JX929742</td>
</tr>
<tr>
<td>G6PD</td>
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<td>TCTGCTAGGTCTGAGCTG</td>
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<tr>
<td>6PGD</td>
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<td>CTTGAGAAGAGAGAGAG</td>
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<td>JX929745</td>
</tr>
<tr>
<td>FAS</td>
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<td>CACCTTCTGGTCACCAACCCT</td>
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</tr>
<tr>
<td>ACCa</td>
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<td>GGCTGTTGATTGGTGGTCTG</td>
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<td>JX929746</td>
</tr>
<tr>
<td>ACCb</td>
<td>GTCTCCATCAGCCCTGTTGGT</td>
<td>CCTGCAGACTCAGCTGAGG</td>
<td>231</td>
<td>JX929747</td>
</tr>
<tr>
<td>LPL</td>
<td>AGGGATGGAGGAGATGCTGTG</td>
<td>TCTGACAGCTGCTGACAGG</td>
<td>173</td>
<td>JX929743</td>
</tr>
<tr>
<td>ATGL</td>
<td>CTGGGTATCGCTCTTGGTGT</td>
<td>TCTGAGAAGAGAGAGAG</td>
<td>291</td>
<td>KF614123</td>
</tr>
<tr>
<td>HSL</td>
<td>GAAGAGAAGAAGAGAAGAAGA</td>
<td>TGTTACCAACAGAGAGAAGG</td>
<td>110</td>
<td>JX929744</td>
</tr>
<tr>
<td>β-Actin</td>
<td>GCACATAGGAAGAAGAGAGAAGA</td>
<td>ACATCTGCTGGAAGAGAAGA</td>
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<td>EU161066</td>
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CPT1A, carnitine palmitoyltransferase 1A; SREBP-1, sterol regulatory element-binding protein-1; G6PD, glucose-6-phosphate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; LPL, lipoprotein lipase; ATGL, adipose TAG lipase; HSL, hormone-sensitive lipase.
that only one PCR product was amplified and that the stock solutions were not contaminated. Standard curves were constructed for each gene using serial dilutions of the stock cDNA to account for any differences in amplification efficiencies. A melting curve was generated for every PCR product confirmed by Oil Red O staining (Fig. 2).

**Statistical analysis**

Results are presented as means with their standard errors. Before statistical analysis, all data were tested for the normality of distribution using the Kolmogorov–Smirnov test. The homogeneity of variances among the different normality of distribution was confirmed by Bartlett’s test. The analysis was carried out using SPSS 10.0 for Windows (SPSS, Inc.), and the minimum significant level was set at 0.05.

**Results**

**Growth performance and body lipid content**

During the experiment, fish were in good health and no mortality was observed (Table 3). There were no significant differences in feed intake among the treatment groups. Compared with the control group, the low-carnitine diet-fed group exhibited higher weight gain and hepatosomatic index, but a lower feed conversion ratio. However, there were no significant differences in weight gain, feed conversion ratio and hepatosomatic index between the control and the high-carnitine diet-fed groups.

Total carnitine content in the liver and muscle was significantly higher in the two carnitine-supplemented groups than in the control group (Fig. 1). Compared with the control diet, the low-carnitine and high-carnitine diets significantly increased lipid content in the liver (Fig. 1). The high-carnitine diet also improved lipid accumulation in muscle (P<0.05). However, there were no significant differences in muscle lipid content between the control and the low-carnitine diet-fed groups. Increased lipid accumulation in the liver as a response to dietary carnitine supplementation was further confirmed by Oil Red O staining (Fig. 2).

**Gene expression involved in lipolysis**

The effects of dietary L-carnitine supplementation on the mRNA levels of lipolytic genes in the liver and muscle of yellow catfish are shown in Fig. 3. Dietary L-carnitine supplementation significantly down-regulated the mRNA levels of CPT1A and PPARα in the liver. The low-carnitine diet also significantly down-regulated the expression of HSL but did not markedly affect that of ATGL mRNA. The high-carnitine diet significantly up-regulated the mRNA levels of both ATGL and HSL.

![Fig. 1. Effects of dietary carnitine concentrations on (A) lipid content and (B) total carnitine (TC) content in the liver and muscle of juvenile yellow catfish (Pelteobagrus fulvidraco). Control diet; low-carnitine diet; high-carnitine diet. Values are means, with their standard errors represented by vertical bars (three replicate tanks and twelve fish were used). Mean values within a tissue with unlike letters were significantly different (P<0.05).](https://doi.org/10.1017/S0007114514001378)
Dietary L-carnitine supplementation reduced the mRNA levels of CPT1A and ATGL in muscle (Fig. 3). The expression of HSL was down-regulated by the high-carnitine diet, but it was not significantly influenced by the low-carnitine diet. Dietary l-carnitine concentrations did not markedly affect the expression of PPARα.

Gene expression involved in lipogenesis

The effects of dietary L-carnitine supplementation on the mRNA levels of LPL and lipogenic genes in the liver and muscle of yellow catfish are shown in Fig. 4. Compared with the control diet, the low-carnitine and high-carnitine diets up-regulated the mRNA levels of SREBP-1, PPARγ, FAS and ACCa.

Fig. 2. (A) Oil Red O staining of the liver tissue sections of yellow catfish (Pelteobagrus fulvidraco) fed the control (■), low-carnitine (▲) and high-carnitine (■) diets. (B) Relative areas stained by Oil Red O were analysed by Image-Pro Plus 6.0 (Media Cybernetics). Values are means (n 4), with their standard errors represented by vertical bars. a,b,c Mean values with unlike letters were significantly different (P<0.05).

Fig. 3. Results of quantitative real-time PCR analysis carried out for genes involved in lipolysis ((A) PPARα, (B) carnitine palmitoyltransferase 1A (CPT1A), (C) hormone-sensitive lipase (HSL) and (D) adipose TAG lipase (ATGL)) in the liver and muscle of yellow catfish (Pelteobagrus fulvidraco) after dietary L-carnitine supplementation for 8 weeks. ■ Control diet; ▲ low-carnitine diet; ■ high-carnitine diet. Values are means, with their standard errors represented by vertical bars (three replicate tanks and twelve fish were used), normalised to β-actin expressed as a ratio of the control (control = 1). a,b,c Mean values within a tissue with unlike letters were significantly different (P<0.05).
In contrast, the expression of G6PD and ACCb remained relatively constant after dietary L-carnitine supplementation. The low-carnitine diet did not significantly affect the expression of LPL and 6PGD. However, the high-carnitine diet significantly up-regulated the mRNA levels of LPL and 6PGD.

Compared with the control diet, the low-carnitine and high-carnitine diets did not significantly affect the mRNA levels of PPARγ and FAS. The low-carnitine diet did not affect the mRNA levels of SREBP-1, LPL and ACCa. In contrast, the high-carnitine diet down-regulated the mRNA levels of SREBP-1 and ACCa and up-regulated the mRNA levels of LPL. Compared with the control diet, the low-carnitine and high-carnitine diets significantly up-regulated the mRNA levels of G6PD and 6PGD.

Fig. 4. Results of quantitative real-time PCR analysis carried out for (C) lipoprotein lipase (LPL) gene and genes involved in lipogenesis ((A) sterol-regulator element-binding protein-1 (SREBP-1), (B) PPARγ, (D) fatty acid synthase (FAS), (E) acetyl-CoA carboxylase a (ACCa), (F) ACCb, (G) glucose-6-phosphate dehydrogenase (G6PD) and (H) 6-phosphogluconate dehydrogenase (6GPD)) in the liver and muscle of yellow catfish (Pelteobagrus fulvidraco) after dietary L-carnitine supplementation for 8 weeks. □, Control diet; ■, low-carnitine diet; ●, high-carnitine diet. Values are means, with their standard errors represented by vertical bars (three replicate tanks and twelve fish were used), normalised to β-actin expressed as a ratio of the control (control = 1). a,b,c Mean values within a tissue with unlike letters were significantly different (P<0.05).
**Enzyme activity**

The effects of dietary l-carnitine supplementation on the activities of LPL and lipogenic enzymes (FAS, G6PD, 6PGD, ME and ICDH) in the liver and muscle of yellow catfish are shown in Fig. 5. Dietary l-carnitine supplementation increased the activities of FAS, G6PD, 6PGD and ME in the liver. The activity of LPL was also enhanced by the low-carnitine diet, but it was not affected by the high-carnitine diet. There were no significant differences in the activity of ICDH between the control and the two carnitine-supplemented groups.

The low-carnitine diet inhibited the activities of G6PD and ICDH in muscle, but did not significantly affect the activities of the other enzymes, such as FAS, LPL, 6PGD and ME (Fig. 5). The high-carnitine diet reduced the activities of almost all the analysed enzymes, except LPL. The activity of LPL was significantly enhanced by the high-carnitine diet.

**Analysis of Pearson’s correlations among several parameters**

Pearson’s correlations among mRNA levels, lipid content and enzyme activity in the liver and muscle of yellow catfish fed diets containing different concentrations of l-carnitine for 8 weeks are summarised in Tables 4 and 5. In the liver, lipid content was positively correlated with the mRNA levels of SREBP-1, PPARγ, FAS and ACCa and the activities of FAS, LPL, G6PD and 6PGD (Table 4). In contrast, it was negatively correlated with the expression of PPARα, CPT1A and HSL. The mRNA levels of SREBP-1 and PPARγ were positively correlated with the expression of FAS and ACCa and the activities of FAS, LPL, G6PD and 6PGD. A positive correlation was also found between the mRNA levels of SREBP-1 and PPARγ. The mRNA levels of PPARα were positively correlated with the expression of CPT1A.

![Fig. 5. Effects of dietary carnitine concentrations on the activities of enzymes (A) fatty acid synthase (FAS), (B) lipoprotein lipase (LPL), (C) glucose-6-phosphate dehydrogenase (G6PD), (D) 6-phosphogluconate dehydrogenase (6PGD), (E) malic enzyme (ME) and (F) isocitrate dehydrogenase (ICDH) involved in lipid metabolism in the liver and muscle of yellow catfish (Pelteobagrus fulvidraco) after dietary l-carnitine supplementation for 8 weeks. □ Control diet; □ low-carnitine diet; □ high-carnitine diet. Values are means, with their standard errors represented by vertical bars (three replicate tanks and twelve fish were used). a,b,c Mean values within a tissue with unlike letters were significantly different (P<0.05).](https://www.cambridge.org/core/journals/br...
In muscle, lipid content was positively related to the mRNA levels of LPL and ACCb and the activities of LPL and 6PGD and negatively related to the expression of ATGL (Table 5). Negative correlations between lipid content and SREBP-1 expression and between lipid content and G6PD and ICDH activities were also detected. The expression of SREBP-1 was positively related to the activities of G6PD and ICDH, while the expression of PPARγ was positively related to that of FAS. A positive relationship was also detected between PPARα and HSL expression.

### Table 4. Correlations* among lipid content, mRNA levels of genes involved in lipid metabolism and activities of several enzymes in the liver of yellow catfish fed diets containing different L-carnitine concentrations for 8 weeks

<table>
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<tr>
<th>Lipid content</th>
<th>SREBP-1</th>
<th>PPARγ</th>
<th>PPARα</th>
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<td></td>
<td>R</td>
<td>P</td>
<td>R</td>
</tr>
<tr>
<td>SREBP-1</td>
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<td></td>
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<tr>
<td>PPARγ</td>
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<tr>
<td>PPARα</td>
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<td>CPT1A</td>
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<td>HSL</td>
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<td>0.01</td>
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<tr>
<td>ATGL</td>
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<td>0.493</td>
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<td>ACCα</td>
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</tr>
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<td>ACCb</td>
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<td>G6PD</td>
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<th>Enzyme activity</th>
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<th>6PGD</th>
<th>ME</th>
<th>ICDH</th>
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<td>Lipid content</td>
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<td>0.003</td>
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<td>PPARγ</td>
<td>0.840</td>
<td>0.005</td>
<td>0.943</td>
<td>0.000</td>
<td>0.926</td>
<td>0.000</td>
</tr>
<tr>
<td>PPARα</td>
<td>0.818</td>
<td>0.007</td>
<td>0.921</td>
<td>0.000</td>
<td>0.845</td>
<td>0.004</td>
</tr>
<tr>
<td>CPT1A</td>
<td>0.442</td>
<td>0.234</td>
<td>0.584</td>
<td>0.099</td>
<td>0.796</td>
<td>0.01</td>
</tr>
<tr>
<td>HSL</td>
<td>0.378</td>
<td>0.015</td>
<td>0.347</td>
<td>0.360</td>
<td>0.541</td>
<td>0.132</td>
</tr>
</tbody>
</table>

### Table 5. Correlations* among lipid content, mRNA levels of genes involved in lipid metabolism and activities of several enzymes in the muscle of yellow catfish fed diets containing different L-carnitine concentrations for 8 weeks

<table>
<thead>
<tr>
<th>Lipid content</th>
<th>SREBP-1</th>
<th>PPARγ</th>
<th>PPARα</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
<td>R</td>
</tr>
<tr>
<td>SREBP-1</td>
<td>0.924</td>
<td>0.000</td>
<td>0.164</td>
</tr>
<tr>
<td>PPARγ</td>
<td>0.131</td>
<td>0.737</td>
<td>0.656</td>
</tr>
<tr>
<td>PPARα</td>
<td>0.665</td>
<td>0.050</td>
<td></td>
</tr>
<tr>
<td>CPT1A</td>
<td>0.291</td>
<td>0.447</td>
<td>0.464</td>
</tr>
<tr>
<td>HSL</td>
<td>0.643</td>
<td>0.062</td>
<td>0.554</td>
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<tr>
<td>ATGL</td>
<td>0.820</td>
<td>0.007</td>
<td>0.861</td>
</tr>
<tr>
<td>LPL</td>
<td>0.848</td>
<td>0.004</td>
<td>0.739</td>
</tr>
<tr>
<td>FAS</td>
<td>0.256</td>
<td>0.506</td>
<td>0.310</td>
</tr>
<tr>
<td>ACCα</td>
<td>0.521</td>
<td>0.151</td>
<td>0.319</td>
</tr>
<tr>
<td>ACCb</td>
<td>0.785</td>
<td>0.012</td>
<td>0.879</td>
</tr>
<tr>
<td>G6PD</td>
<td>0.501</td>
<td>0.170</td>
<td>0.546</td>
</tr>
<tr>
<td>6PGD</td>
<td>0.455</td>
<td>0.219</td>
<td>0.513</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>LPL</th>
<th>FAS</th>
<th>G6PD</th>
<th>6PGD</th>
<th>ME</th>
<th>ICDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid content</td>
<td>0.807</td>
<td>0.009</td>
<td>0.773</td>
<td>0.015</td>
<td>0.614</td>
<td>0.078</td>
</tr>
<tr>
<td>SREBP-1</td>
<td>0.131</td>
<td>0.737</td>
<td>0.509</td>
<td>0.161</td>
<td>0.486</td>
<td>0.185</td>
</tr>
<tr>
<td>PPARγ</td>
<td>0.693</td>
<td>0.000</td>
<td>0.891</td>
<td>0.001</td>
<td>0.294</td>
<td>0.442</td>
</tr>
<tr>
<td>PPARα</td>
<td>0.820</td>
<td>0.007</td>
<td>0.717</td>
<td>0.030</td>
<td>0.436</td>
<td>0.241</td>
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<td>CPT1A</td>
<td>0.756</td>
<td>0.018</td>
<td>0.839</td>
<td>0.005</td>
<td>0.931</td>
<td>0.000</td>
</tr>
<tr>
<td>HSL</td>
<td>0.798</td>
<td>0.01</td>
<td>0.676</td>
<td>0.045</td>
<td>0.394</td>
<td>0.294</td>
</tr>
<tr>
<td>ATGL</td>
<td>0.264</td>
<td>0.493</td>
<td>0.108</td>
<td>0.783</td>
<td>0.223</td>
<td>0.564</td>
</tr>
<tr>
<td>LPL</td>
<td>0.312</td>
<td>0.413</td>
<td>0.221</td>
<td>0.567</td>
<td>0.034</td>
<td>0.931</td>
</tr>
<tr>
<td>FAS</td>
<td>0.782</td>
<td>0.017</td>
<td>0.855</td>
<td>0.003</td>
<td>0.978</td>
<td>0.000</td>
</tr>
<tr>
<td>ACCα</td>
<td>0.964</td>
<td>0.000</td>
<td>0.956</td>
<td>0.000</td>
<td>0.798</td>
<td>0.000</td>
</tr>
<tr>
<td>ACCb</td>
<td>0.453</td>
<td>0.221</td>
<td>0.467</td>
<td>0.205</td>
<td>0.156</td>
<td>0.688</td>
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<tr>
<td>G6PD</td>
<td>0.46</td>
<td>0.213</td>
<td>0.162</td>
<td>0.677</td>
<td>0.044</td>
<td>0.91</td>
</tr>
<tr>
<td>6PGD</td>
<td>0.167</td>
<td>0.667</td>
<td>0.014</td>
<td>0.972</td>
<td>0.264</td>
<td>0.493</td>
</tr>
</tbody>
</table>

*Positive R and P < 0.05 indicate the positive correlation between the two variables; negative R and P < 0.05 indicate the negative correlation between the two variables; IP address: 54.70.40.11, on 25 Oct 2019 at 11:26:38, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
Discussion

In the present study, a growth-promoting effect of the reasonable levels of L-carnitine supplementation was found in yellow catfish, in agreement with many other reports\(^5\)\(^-\)\(^7\)\(^,\)\(^11\)\(^,\)\(^29\)\(^,\)\(^29\). It was also found that high dietary carnitine concentrations (3395 mg/kg diet) did not promote growth performance. Similarly, Keshavanath & Renuka\(^7\) reported that 0·5 g/kg carnitine (3395 mg/kg diet) did not promote growth performance. Similarly, L-carnitine supplementation was found to up-regulate the mRNA levels of several lipolytic genes (CPT1A, ATGL and HSL), in agreement with the increased lipid content in muscle. The present study also demonstrated that carnitine supplementation up-regulated the mRNA levels of G6PD and G6PD. However, the activities of G6PD and G6PD declined with carnitine supplementation. Similarly, enzyme activities were not found to be always accompanied by parallel changes in mRNA levels in several studies\(^3\)\(^,\)\(^4\)\(^,\)\(^5\)\(^,\)\(^6\)\(^,\)\(^8\)\(^,\)\(^9\). Studies have indicated that gene expression is affected by mRNA stability\(^3\)\(^6\) and also that it is time course dependent\(^3\)\(^7\). The increase in LPL activity and mRNA levels might indicate an increase in the import of lipids into muscle for storage. In addition, the increase in ACC\(b\) expression might, in turn, inhibit CPT1 activity through its malonyl-CoA product\(^3\)\(^8\)\(^,\)\(^9\). These findings could, to some extent, explain the increase in lipid accumulation in the muscle of yellow catfish fed the high-carnitine diet. The positive relationships between lipid content and the parameters mentioned above further confirm this concept.

PPAR\(\alpha\) and PPAR\(\gamma\) are two key transcription factors that are involved in lipid metabolism\(^4\)\(^,\)\(^4\)\(^1\). PPAR\(\alpha\) plays key roles in the catabolism of fatty acids by up-regulating the expression of several key enzymes involved in fatty acid oxidation\(^4\)\(^2\)\(^,\)\(^4\)\(^3\), while PPAR\(\gamma\) is critical for the regulation of lipogenesis and promotes lipid storage\(^4\)\(^,\)\(^4\)\(^4\). In the present study, dietary carnitine supplementation was found to down-regulate PPAR\(\alpha\) mRNA levels but to up-regulate PPAR\(\gamma\) mRNA levels in the liver, which again correlated well with the reported increase in lipid content in the liver. Furthermore, the mRNA levels of PPAR\(\gamma\) in the liver were positively correlated with those of FAS and ACC\(a\) and with the activities of several lipogenic enzymes (such as FAS, G6PD, G6PD and ME) and were negatively correlated with the mRNA levels of CPT1A. The mRNA levels of PPAR\(\alpha\) were positively correlated with those of CPT1A and negatively correlated with those of FAS and ACC\(a\) and with the activities of lipogenic enzymes (such as FAS, G6PD, G6PD and ME). All these findings support the importance of PPAR\(\alpha\) and PPAR\(\gamma\) in the regulation of lipid metabolism, in agreement with those of the other studies\(^5\)\(^,\)\(^5\)\(^,\)\(^4\)\(^5\)\(^,\)\(^4\)\(^6\). Studies have also suggested that PPAR\(\alpha\) is stimulated through a PPAR response element in the first and second introns of the human and rat CPT1A genes, respectively\(^4\)\(^7\)\(^,\)\(^4\)\(^8\). SREBP-1 is a membrane-bound transcription factor that regulates the gene expression of enzymes involved in fatty acid synthesis\(^4\)\(^9\). In the present study, increased mRNA levels of SREBP-1 were observed in the liver of fish fed the carnitine-supplemented diets, in agreement with the increase in lipid content in the liver. However, a negative
correlation was observed between lipid content and SREBP-1 mRNA levels in muscle. The reason for this is not known; however, it might be due to the tissue-specific role of SREBP-1 in the regulation of lipid metabolism.

In conclusion, in contrast to our hypothesis, dietary carnitine supplementation was found to increase lipid content in both the liver and muscle in the present study. The increased lipid content in the liver could be attributed to the up-regulation of the mRNA levels of lipogenic genes (such as SREBP, PPARY, FAS and ACCa) and to the increased activities of lipogenic enzymes (such as FAS, G6PD, 6PGD and ME) and to the down-regulation of the mRNA levels of the lipolytic gene CPTIA. The increased lipid content in muscle could be attributed to the down-regulation of the mRNA levels of lipolytic genes (CPTIA and ATGL) and to the increased activity of LPL. Thus, dietary carnitine influenced lipid deposition by a tissue-specific mechanism, probably through different lipid metabolic strategies occurring as a result of competitions between lipolysis and lipogenesis and between export and import of lipids from different tissues. To our knowledge, the present study is the first to explore the effect of dietary carnitine on the growth performance in fingerlings of the African catfish (Clarias gariepinus) in relation to dietary lipid. Br J Nutr 69, 289–299.


