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Dietary lipid concentration affects liver mitochondrial DNA copy number, gene expression and DNA methylation in large yellow croaker (*Larimichthys crocea*)

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ABSTRACT

In response to changes in energy demand and nutrient supply, the organism regulates mitochondrial metabolic status to coordinate ATP production. To survey mitochondrial metabolic adaptation in response to dietary lipid concentration, citrate synthase (EC 2.3.3.1, CS) activity, the expression of several mitochondrial transcription factors, mitochondrial DNA (mtDNA) copy number, mitochondrial gene expression, mtDNA methylation, and oxidative stress parameters were analyzed in the liver of large yellow croaker fed one of three diets with a low (6%), moderate (12%, the control diet) or high (18%) crude lipid content for 70 d. MtDNA copy number was significantly increased in the low- and high-lipid groups compared to the control. The transcription of cytochrome *c* oxidase 1 (*COX1*), *COX2*, *COX3*, ATP synthase 6 (*ATPase 6*), *12S rRNA* and *16S rRNA* was also significantly increased in the low-lipid group compared with the control, while the transcription of these genes in the high-lipid group was unchanged. Moreover, *D-loop* (displacement loop) methylation in the high-lipid group was significantly higher than the control. The increase in mtDNA copy number and mitochondrial transcription might be a compensatory mechanism that matches ATP supply to demand under a low-lipid diet, while the increase of mtDNA copy number with unchanged mitochondrial transcription in the high-lipid group probably came from the increase of *D-loop* methylation.

1. Introduction

Physiological, developmental, or environmental factors change the status of energy demand and supply. In response to changes in energy demand and supply, the organism regulates mitochondrial metabolic status to coordinate ATP production (Bremer et al., 2012). In fish, mitochondrial metabolic adaptation and its mechanisms in response to temperature (Battersby and Moyes, 1998; Hardewig et al., 1999; Lucassen et al., 2003; LeMoine et al., 2008; O'Brien, 2011; Bremer et al., 2012; Dos Santos et al., 2012), photoperiod (Martin et al., 2009) and exercise (McClelland et al., 2006; LeMoine et al., 2010) were widely studied. Mitochondrial metabolic adaptation in response to these stimuli can be achieved by changes in mitochondrial volume density (Urschel and O'Brien, 2008), mitochondrial membrane phospholipid fatty acid composition (Kraffe et al., 2007), mitochondrial enzyme activities (Hardewig et al., 1999; Lucassen et al., 2006; Duggan et al., 2011), mitochondrial oxidative capacity (Dos Santos et al., 2012), mitochondrial transcription factors expression (Bremer et al., 2012), mitochondrial DNA (mtDNA) copy number (Battersby and Moyes, 1998; Hardewig et al., 1999) and mitochondrial mRNA abundance (Battersby and Moyes, 1998). Dependent of these changes, oxygen and ROS balance may also be altered (Heise et al., 2007; Grim et al., 2010; Kammer et al., 2011). Although there are a myriad of pathways to coordinate mitochondrial metabolic adaptation in response to stimuli, the pathway appears versatile in species, tissue and stimuli type (O'Brien, 2011). For instance, two subspecies of killifish have different ability to increase citrate synthase activity and mitochondrial volume and surface densities at colder temperatures (Dhillon and Schulte, 2011). The activities of citrate synthase (EC 2.3.3.1, CS) and cytochrome-c oxidase (EC 1.9.3.1, COX) increased in both liver and muscle of threespine sticklebacks in response to cold acclimation, but increased mitochondrial volume density only occurred in muscle (Orczewska et al., 2010). The activities of CS and COX increased in response to both temperature and exercise in zebrafish, yet only cold acclimatization increased β-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) and pyruvate kinase

Abbreviations: ATPase 6, ATP synthase 6; COX, Cytochrome c oxidase; CS, Citrate synthase; CYTB, Cytochrome b; D-loop, Displacement loop; HIF1α, Hypoxia-inducible factor 1α; mtDNA, Mitochondrial DNA; MDA, Malondialdehyde; ND6, NADH dehydrogenase subunit 6; ND2, NADH dehydrogenase subunit 2; ND4L, NADH dehydrogenase 4 L; NRF1, Nuclear respiratory factor 1; PGC1α, Peroxisome proliferator-activated receptor gamma coactivator-1-α; PPAR, Peroxisome proliferator-activated receptor; rRNA, Ribosomal RNA; SOD, Superoxide dismutase activity; 5mC, 5-Methylcytosine; 8-OHdG, 8-Hydroxydeoxyguanosine.

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Table 1
Ingredient composition of the experimental diets[a].

Ingredients (g/100 g)	Dietary lipid concentration (%)		
	Low (6)	Moderate (12)	High (18)
Fish meal[b]	39	39	39
Soybean meal[b]	20	20	20
Wheat meal[b]	23.3	23.3	23.3
Wheat starch[b]	12	6	0
Fish oil[b]	0	6	12
Soybean lecithin[b]	1.5	1.5	1.5
Vitamin premix[c]	2	2	2
Mineral premix[d]	2	2	2
Attractant[e]	0.1	0.1	0.1
Mold inhibitor[f]	0.1	0.1	0.1
Proximate composition (g/100 g)			
Moisture	9.5	9.4	9.2
Crude protein	43.1	42.6	43.2
Crude lipid	6.1	11.5	17.8

[a] Referred to Yan et al. (Yan et al., 2015).
[b] All of these ingredients were supplied by Great Seven Biotechnology Co., Ltd., China.
[c] Vitamin premix (mg or g/kg diet): cholecalciferol, 5 mg; retinol acetate, 32 mg; thiamin 25 mg; vitamin B_{12} (1%), 10 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; ascorbic acid, 2000 mg; alpha-tocopherol (50%), 240 mg; vitamin K_3, 10 mg; pantothenic acid, 60 mg; inositol, 800 mg; niacin acid, 200 mg; folic acid, 20 mg; biotin (2%), 60 mg; choline chloride (50%), and 4000 mg; microcrystalline cellulose, 12.47 g.
[d] Mineral premix (mg or g/kg diet): $CuSO_4 \cdot 5H_2O$, 10 mg; Ca $(IO_3)_2 \cdot 6H_2O$ (1%), 60 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $FeSO_4 \cdot H_2O$, 80 mg; $MgSO_4 \cdot 7H_2O$, 1200 mg; $MnSO_4 \cdot H_2O$, 45 mg; $NaSeSO_3 \cdot 5H_2O$ (1%), 20 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $CaH_2PO_4 \cdot H_2O$, and 10 g; zeolite, 8.485 g.
[e] Attractants: glycine and betaine.
[f] Mold inhibitor: contained 50% calcium propionic acid and 50% fumaric acid.

(EC 2.7.1.40) (McClelland et al., 2006). Although mitochondrial metabolic adaptation and its versatility in response to temperature, exercise and photoperiod have been widely examined, few studies have examined mitochondrial metabolic adaptation in response to diet in fish (LeMoine et al., 2008).

Changes in diet have a pronounced effect on the tissue-specific metabolic strategy and mitochondrial phenotypes in most vertebrate species (Blasco et al., 1992; Ojano-Dirain et al., 2005; Chanseaume et al., 2007). In mammals, mitochondrial proliferation is triggered by dietary restrictions (Civitarese et al., 2007). Meanwhile, several studies on mammals indicated that high-lipid diet increases mitochondrial content to maintain normal respiratory function as a possible response to an increased lipid overload (Hancock et al., 2008; Carabelli et al., 2011; Ruggiero et al., 2011). In fish, dietary nutrient density was associated with mitochondrial function such as mitochondrial gene expression and mitochondrial respiratory chain enzyme activities (Eya et al., 2011, 2012). Further study on the mitochondrial metabolic adaptation of goldfish to dietary lipid has suggested that increases in aerobic metabolic capacity (CS) may not always coincide with mitochondrial biogenesis (COX) in the liver, and nuclear respiratory factor 1 (*NRF1*) and peroxisome proliferator-activated receptors (*PPARs*) were involved in the regulation of mitochondrial gene expression and fatty acid oxidation gene expression (LeMoine et al., 2008). However, it is unclear how dietary lipid concentration affects qualitative aspects of mitochondrial metabolism, such as mtDNA copy number and mitochondrial gene expression. In addition, whether changes in ROS balance are involved in metabolic adaptation in response to dietary lipid concentration in fish remains unknown. Current evidence suggests that mitochondria are susceptible to ROS (Kujoth et al., 2005), a mediator of DNA methylation (Franco et al., 2008), but no studies have investigated whether changes in ROS metabolism affect mtDNA such as mtDNA methylation in response to dietary lipid concentration.

The goals for this study were to determine: (1) how large yellow croaker *Larimichthys croceus*, one of the most important mariculture fish species in China, coordinate mtDNA copy number and mitochondrial gene expression in responses to changes of dietary lipid concentration; (2) whether changes in ROS balance are involved in metabolic adaptation in response to changes of dietary lipid concentration; and

Table 2
Primers used in this study[a].

Accession	Gene	Forward	Reverse
Quantitative real-time PCR primer			
KM593915	*HIF1α*	GGAAGGTGCTCCACTGCT	TATGGCGGCTGAGGAAG
KM593916	*NRF1*	GTGCCGTCTCAAACTGTGG	GTGCCAACCTGGATGAGC
KM593914	*PGC1α*	CTGCTCAGTATGGCAACGA	GGTCACTGGCATTGGTCAC
KF998577	*PPARα*	GTCAAGCAGATCCACGAAGCC	TGGTCTTTCCAGTGAGTATGAGCC
GU584189	*β-Actin*	CTACGAGGGTTATGCCCTGCC	TGAAGGAGTAACCGCGCTCTGT
XM_010751754	*Ubiquitin*	TGGAGGATGGACGCACACTG	GCAGACGGGCATAGCACTTG
GQ168793	*β-ActinNDA*	CCCAACTTGAGCCTAACAT	TACCTCCAGACAGCACGG
EU339149	*12S r RNA*	ACAACCAACCATAGCCCACA	GTGGCTGGCACGAGTTTGA
EU339149	*ND4L*	CTTCTCCGCAGCCTTCATT	GCGATGAATAGGGAAAGCA
EU339149	*D-loop*	CTGAGGTTGGTGGAGTGC	GGGTTGCTCCCACTTATGT
EU339149	*16S r RNA*	TATGAATGGCAAGACGAGG	TAGGACAGGGCTCAGTTAGTT
EU339149	*ND2*	GACCTCATTACAGGACTTATCAT	TGTAGGACGAGGATTATTCAG
EU339149	*ND3*	CTATGAGTGCGGCTTTGAC	AAGGTAAGGAGAAGCAGGAC
EU339149	*ND6*	ATGTTGGTGGTGTTTGCG	CCTCGCAATACAGATAACTCC
EU339149	*COX1*	CCTGCTGCTCTCACTACCTG	CCGAAGAATCAGAATAGGTGTT
EU339149	*COX2*	GAGTGCTAATCTCCGCTGAAG	TGGGACTGCTTCAACTACGAT
EU339149	*COX3*	ACTTCCACTCTACAATCCTCCTAT	AGAAGACCTCTGATGTGATGAAT
EU339149	*ATPase6*	ATTAGCGATTGCTCTCATACT	CGAGTATTAGGGCTCAGTTAT
EU339149	*CYTB*	GCCTCTACTATGGCTCCTATCTT	AGGCACTGCTGACAAGAGGT
Bisulfite-pyrosequencing primer			
EU339149	*D-loop*-F	GGGATATTGATTGATAATTATTTGG	
	D-loop-R(bio)	ACGACRACCTTATACCTAAATACCTC	
	D-loop-sp	GGTATTTTTTTATTTTGATTG	
EU339149	*12S*-F	ATGAGTTGAATAGGCGATTAGTTTA	
	12S-R(bio)	TACTAAACCTACTAATCCTAAAATAAAAACTAC	
	12S-sp	ATTTGATTTYGGTTTAAAAG	
EU339149	*ND6*-F	TTATTAATATTAGTTTTGATAATTTTAGTGTT	
	ND6-R(bio)	AATATTAATAATTAATACTTAAATATTACTTTTAAC	
	ND6-sp	GATAATTTTAGTGTTYGTTTTTTAA	

[a] *ATPase6*, ATP synthase; *COX*, cytochrome *c* oxidase; *CYTB*, cytochrome b; *HIF1α*, hypoxia-inducible factor 1α; *ND*, NADH dehydrogenase; *NRF1*, nuclear respiratory factor 1; *PGC1α*, peroxisome proliferator-activated receptor gamma coactivator 1α; and *PPAR*, peroxisome proliferator-activated receptor. All sequences available from the GenBank database (www.ncbi.nlm.nih.gov).