

Article

Comparative Transcriptome Analysis of Orbital Fat Reveals Stage-Specific Gene Expression Associated with Growth Variation in Bighead Carp (*Hypophthalmichthys nobilis*)

Junru Wang ^{1,2}, Qi Lei ^{1,2}, Jun Liu ^{1,2}, Haijun Tian ^{1,2}, Gaoyou Yao ^{1,2}, Zhiruo Sun ^{1,2}, Xusheng Guo ^{1,2,*} and Jingou Tong ^{3,*} 

¹ School of Fisheries, Xinyang Agriculture and Forestry University, Xinyang 464000, China; jrwang1021@163.com (J.W.)

² Fishery Biological Engineering Technology Research Center of Henan Province, Xinyang 464000, China

³ State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Academy of Seed Design, Chinese Academy of Sciences, Wuhan 430072, China

* Correspondence: 2015210001@xyafu.edu.cn (X.G.); jgtong@ihb.ac.cn (J.T.)

Simple Summary

Bighead carp is a valuable farmed fish, and the fat behind its eyes, known as orbital fat, is a prized part of the head. While developmental changes in orbital fat have been described, the molecular basis for growth variation among individuals of the same age remains unclear. Transcriptional profiles were compared in orbital fat from extreme growth phenotypes at juvenile (6-month) and market-size (18-month) stages. In juveniles, slow growth was associated with upregulation of stress-related genes (e.g., *sgk1*, *fkbp5*), whereas in market-size fish, fast growth correlated with lipid-storage genes (e.g., *dgat2*), and slow growth with lipid-oxidation genes (e.g., *cpt1b*). These findings suggest stage-dependent regulatory roles of orbital fat in growth. The candidate genes identified provide potential molecular markers for selective breeding aimed at improving growth performance in bighead carp.

Abstract

Bighead carp (*Hypophthalmichthys nobilis*) is a key aquaculture species, with the head and its orbital fat being a commercially valuable product. To elucidate the molecular basis of growth variation, we performed comparative transcriptome analysis of orbital fat from extreme growth phenotypes at juvenile (6 months) and market-size (18 months) stages. In juveniles, slow growth was linked to upregulation of stress-responsive genes (*sgk1*, *fkbp5*, *lipg*), while fast growth correlated with higher expression of stress-buffering (*crhbp*) and nutrient-signaling (*rbp2*, *mgea5*) genes. At 18 months, divergent growth aligned with opposing lipid metabolic states: a pro-anabolic profile (*dgat2*, *fads2*) supported fast growth, whereas a catabolic profile (*cpt1b*, *ppargc1a*) was associated with slow growth. These results demonstrate stage-specific transcriptional reprogramming in orbital fat underlying growth variation. This study provides a molecular framework for orbital fat-mediated growth regulation and highlights potential candidate genes for molecular breeding in bighead carp.

Keywords: bighead carp; orbital fat; growth variation; transcriptome; differentially expressed genes (DEGs)



Academic Editor: Jingxia Liu

Received: 4 February 2026

Revised: 25 February 2026

Accepted: 28 February 2026

Published: 4 March 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons](https://creativecommons.org/licenses/by/4.0/)

[Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Adipose tissue is the primary energy storage organ in fish, supplying essential fatty acids and serving as a carrier for fat-soluble vitamins, thereby playing a critical role in piscine nutrition and physiology [1]. Beyond lipid storage and metabolism, it also functions as an endocrine organ, secreting adipokines that contribute to the systemic regulation of lipid metabolism. Lipid deposition in fish results from a dynamic balance among dietary lipid intake, de novo lipogenesis, and fatty acid β -oxidation [2]. Teleost fish deposit lipids in diverse sites, including visceral organs, liver, muscle, brain, and other tissues. The extent of this accumulation is influenced by age, genetics, and nutritional status [3].

Lipids serve as an excellent source of metabolic energy and provide essential fatty acids that can support optimal growth rates in fish. The metabolism of these lipids, including adipogenesis, lipolysis, and β -oxidation, is a dynamic and tightly regulated process. It is controlled by a complex interplay of hormones, growth factors, enzymes, and transcription factors. A key aspect of this metabolic capability is the biosynthesis of long-chain polyunsaturated fatty acids (LC-PUFAs). This process is governed not only by the genetic complement of desaturase and elongase enzymes but also by their transcriptional and post-transcriptional expression [4]. Notably, lipid accumulation is intrinsically linked to somatic growth. In Atlantic salmon, lipid content shows a positive correlation with increasing body size [5]. Somatic growth underpins the progression through larval, juvenile, and adult stages, and growth rate is one of the most important economic traits in aquaculture [6]. This trait is regulated by a combination of environmental and genetic factors [7]. The transcriptome represents the complete set of transcripts in a cell or tissue under specific conditions, and its analysis allows for the identification of key genes and pathways underlying phenotypic variation [8]. RNA-seq is a powerful tool for transcriptome characterization in fish. It has greatly advanced our understanding of diverse biological processes, from development and evolution to immune and stress responses [9].

Fish possess the ability to synthesize LC-PUFAs, especially nutritionally important omega-3 highly unsaturated fatty acids (n-3 HUFAs), via fatty acyl desaturases (*Fads*) and elongases (*Elovl*) [10]. Long-chain omega-3 PUFAs such as EPA and DHA are crucial for development and cardiometabolic health. Since humans cannot synthesize them, dietary sources such as fish are essential [11]. Orbital fat is a structural fat located behind the eyeball, functioning to cushion and protect the optic nerve. This tissue is rich in highly unsaturated fatty acids, particularly DHA. In several marine species, such as tuna and skipjack, orbital fat has been identified as an important reservoir of these valuable lipids [12]. This potential is not limited to marine fish. Analyses of freshwater species have revealed that in the planktivorous bighead carp, the brain and ocular tissues (encompassing the orbital fat) contain comparatively higher levels of EPA and DHA [13].

The bighead carp (*Hypophthalmichthys nobilis*) is a vital species in Chinese pond culture, owing to its desirable traits such as fast growth rate, strong stress resistance, and economical production [14]. Its plankton-filtering habit also contributes to water quality regulation [15]. The species is characterized by a large head, which comprises about one-third of its body length and has an edible yield of 67.3%. In contrast to most fish, the head carries a higher market value than the body, largely due to its favorable lipid profile and taste [16]. Notably, the orbital fat is considered a culinary delicacy. Interestingly, the proportion of EPA and DHA in the head of bighead carp does not differ significantly from that of Atlantic salmon [17]. While visceral and intramuscular adipose tissues have been extensively studied in fish, the orbital fat depot, despite its economic importance, remains largely unexplored. In bighead carp, the head, and particularly the orbital fat, commands high market value due to its lipid profile and texture [16]. However, whether this depot plays any role in somatic growth or simply reflects systemic energy status is unknown.

Given its anatomical location and enrichment in DHA [13,17], we hypothesized that the transcriptional state of orbital fat might vary with growth performance, either as a consequence of differential energy allocation or as a local modulator of growth-related processes. This study therefore aimed to characterize the orbital fat transcriptome in extreme growth phenotypes, providing the first investigation of how this economically important tissue differs between fast- and slow-growing individuals.

Our preliminary transcriptomic study compared juvenile and market-size bighead carp and revealed significant transcriptional changes during orbital fat growth, highlighting pathways such as cardiac muscle contraction, PPAR signaling, and glycolysis/gluconeogenesis [18]. This work demonstrated stage-dependent gene regulation in orbital fat development. However, the molecular basis for the pronounced growth variation among individuals of the same age remains unexplored. In the present study, we therefore compared extreme growth phenotypes at both the juvenile and market-size stages to identify stage-specific transcriptional signatures in orbital fat associated with divergent growth rates. The candidate genes identified through this approach may serve as potential molecular markers for selective breeding programs aimed at improving growth performance in bighead carp.

2. Materials and Methods

2.1. Experimental Materials and Sampling

Orbital fat samples were collected from a mixed-family population of bighead carp reared at the Zhangdu Lake Fish Farm (Xinzhou District, Wuhan, China). The experimental population was derived from a controlled mass spawning event involving 10 male and 10 female broodstock. A subset of the offspring was transferred to and reared in a common earthen pond under identical conditions. All fish were maintained in the same pond (area 2000 m², depth 2.5 m) equipped with aeration systems to maintain adequate dissolved oxygen levels and fed a commercial diet (Tongwei 131, Tongwei Co., Ltd., Chengdu, China; crude protein $\geq$ 30%) twice daily (08:00 and 16:00) at 3% of body weight, adjusted monthly based on estimated biomass. Water quality parameters (temperature, dissolved oxygen, pH, ammonia) were monitored weekly and maintained within optimal ranges. To investigate divergent growth phenotypes, approximately 200 fish were sampled at each developmental stage, and body weight was measured individually. The top and bottom 10% of the body weight distribution were selected as the high-body-weight (B) and low-body-weight (S) groups, respectively, ensuring extreme phenotypic contrast. At 6 months post-hatching, these comprised a high-body-weight group (M6B, 512 $\pm$ 4.25 g) and a low-body-weight group (M6S, 114 $\pm$ 3.61 g). Similarly, at the commercial market size of 18 months, a high-body-weight (M18B, 2867 $\pm$ 36.54 g) and a low-body-weight group (M18S, 2189 $\pm$ 34.23 g) were selected. Three biological replicates were included for each group at both time points (total n = 12). Following anesthesia with ethyl 3-aminobenzoate methanesulfonate (MS-222, Sigma-Aldrich, St. Louis, MO, USA), the orbital fat from both eyes of each fish was rapidly and precisely dissected under a stereomicroscope. Care was taken to remove any visible extraocular muscle or connective tissue, ensuring tissue accuracy. Samples from both eyes of the same individual were pooled into one sample per fish, immediately snap-frozen in liquid nitrogen, and stored at -80 °C until RNA extraction.

2.2. RNA Extraction, Quality Assessment, and Sequencing

Total RNA was extracted from all orbital fat samples using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Residual genomic DNA was removed by treatment with RNase-free DNase I (TaKaRa, Dalian, China). RNA integrity was initially verified by 1% agarose gel electrophoresis, while purity and concentration were

determined using a Nanodrop2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Further quality assessment was performed with an Agilent 2100 Bioanalyzer System using the RNA 6000 Nano Kit (Agilent Technologies, Santa Clara, CA, USA). Only RNA samples meeting quality thresholds were used for subsequent library construction. For RNA-seq, 1 µg of total RNA per sample was used for library preparation. Sequencing libraries were prepared and sequenced by Biomarker Technologies Co., Ltd. (Beijing, China) on an Illumina NovaSeq 6000 platform, generating 150 bp paired-end reads for transcriptome analysis.

2.3. Transcriptomic Data Processing and Analysis

Raw sequencing reads were subjected to quality control, including adapter trimming and removal of low-quality sequences, to obtain high-quality clean reads for all downstream analyses. The resulting high-quality clean reads were aligned to the bighead carp reference genome (NCBI BioProject: PRJNA1090045) using HISAT2 (v2.2.1) with default parameters. The resulting Sequence Alignment/Map (SAM) files were converted to sorted and indexed BAM files using SAMtools (v1.12). Only uniquely mapped reads were retained for quantification to minimize potential confounding from multi-mapping reads that could arise from paralogous gene copies, a common feature in teleost genomes due to whole-genome duplication. Subsequently, gene-level counts were obtained from uniquely mapped reads using featureCounts (Subread v2.0.3).

The raw count matrix served as input for differential expression analysis, which was conducted in R with the DESeq2 package (v1.30.1). For visualization and interpretation purposes, expression levels were also quantified as Fragments Per Kilobase of exon per Million mapped fragments (FPKM). The False Discovery Rate (FDR) was controlled using the Benjamini–Hochberg method for multiple testing correction. Genes with an FDR-adjusted p -value ≤ 0.01 and an absolute fold change ≥ 2 were defined as significantly differentially expressed (DEGs).

Functional enrichment analysis of Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was conducted using the clusterProfiler R package (v4.4.1). FDR correction was applied simultaneously across all terms within the three GO domains (Biological Process, Cellular Component, Molecular Function) and KEGG pathways. To visualize sample relationships and assess the reproducibility of biological replicates, principal component analysis (PCA) was performed on the variance-stabilizing transformation (vst) of the count data using the plotPCA function in the DESeq2 package (v1.30.1). PCA was conducted separately for each developmental stage (6 months and 18 months) using all expressed genes. The resulting plots were generated with custom R scripts using the ggplot2 package (v3.3.5), with points colored by growth phenotype (B vs. S).

2.4. Quantitative Real-Time PCR (qRT-PCR) Confirmation of RNA-Seq Expression Profiles

To validate the RNA-seq results, a subset of DEGs was selected for qRT-PCR analysis. The selection criteria were tailored to each developmental stage to ensure a representative and functionally relevant assessment. For the 6-month-old juveniles, eight genes were chosen primarily based on the significance of differential expression (p -value and fold-change) and their putative involvement in biological processes relevant to early growth, such as stress response and metabolic regulation. For the 18-month-old market-size fish, eight genes were selected to represent a spectrum of fold-change values and to cover key, significantly enriched KEGG pathways, namely carbon metabolism, amino acid biosynthesis, and glycolysis/gluconeogenesis.

For all qRT-PCR assays, β -actin was used as the internal reference gene for normalization. The stability of β -actin was previously validated in our laboratory across different tissues and developmental stages in bighead carp using the geNorm algorithm (M-value < 0.5), confirming its suitability as a reference gene. Total RNA for qRT-PCR was derived from the same biological samples as those used for RNA sequencing. cDNA was synthesized from 1 μ g of total RNA per sample using the PrimeScript™ RT reagent Kit (TaKaRa, Dalian, China) in a final volume of 20 μ L. The resulting cDNA was diluted 1:5 with nuclease-free water prior to qPCR analysis. For qRT-PCR analysis, gene-specific oligonucleotide primers were designed employing Primer 5.0 software (sequences detailed in Table 1). qRT-PCR was performed on a StepOne™ Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) with a 20 μ L reaction mixture containing 10 μ L of SYBR Green Master Mix, 0.6 μ L of each primer (10 μ mol/L), 2 μ L of the diluted cDNA (equivalent to 20 ng of input RNA) and 6.8 μ L of nuclease-free water. The thermal cycling protocol consisted of an initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 45 s. All samples were analyzed with three biological replicates (the same individuals used for RNA-seq) and two technical replicates each. Relative expression was quantified by the $2^{-\Delta\Delta CT}$ method, with β -actin as the reference [19].

Table 1. Primer sequences used for qRT-PCR validation of DEGs.

Primers	Sequence	Tm (°C)	Product Length (bp)
Hyn-sgk1-F	CCCAATGGAACGACATCAAC	58	116
Hyn-sgk1-R	TACAGCACTGCACCCAAACA		
Hyn-rbp2-F	ACCAAACTCTCAGCACCTT	55	148
Hyn-rbp2-R	CTCCCTTCTGGACACAAATC		
Hyn-fkbp5-F	TTTCAAGGCTGGACGGTACA	58	142
Hyn-fkbp5-R	CAATGCGAGATTGAGATGGG		
Hyn-crhhp-F	ATTGATGGCTGGGTATGA	60	246
Hyn-crhhp-R	AAACTGCCCTCTGGGGTCTG		
Hyn-foxo1-F	GTCAGTTCAAAACCCTACCT	55	103
Hyn-foxo1-R	TTCCAGTCTTCTTCCCAATA		
Hyn-nr4a3-F	CGGAGTACAGAGCCTGGTAAA	58	121
Hyn-nr4a3-R	TGGAGATAAAGGGATGGAAAA		
Hyn-pdk2-F	GACCAATGACAGCATTTCCT	58	148
Hyn-pdk2-R	AACACCCACTTCAGCAGCAC		
Hyn-acsm3-F	AGAAGGAATGGGGACAGAAAA	60	147
Hyn-acsm3-R	TGCAGGCGAGCTGAAGGACTA		
Hyn-fads2-F	AGCACGACTTCGGTCATCTATC	55	198
Hyn-fads2-R	GCACAGTTCCTACTACAAACG		
Hyn-fbp2-F	TAGTCTTCTATCCCTTTTCGCA	55	233
Hyn-fbp2-R	CCTATTGTGTTGTCTCTTGGC		
Hyn-cpt1b-F	AGTGCTATGACAGGTGGTTTG	58	219
Hyn-cpt1b-R	ATTGTAGTCTGGTAGGGGGTG		
Hyn-ak1-F	CTCCAGCTCTGCTGCTGTAC	58	116
Hyn-ak1-R	ACGCTTCTTGATGGTCTCTCT		
Hyn-pklr-F	TTATCCCAGCCTAACCCACG	60	148
Hyn-pklr-R	CACGCCCTTACGACTTCCCA		
Hyn-gys1-F	CACTTACTAAAGCGGAAACC	55	188
Hyn-gys1-R	CCAGCAATAAAAAGAAACAC		
Hyn-pygm-F	TTGAGGAGGGCGGACAGAAG	60	176
Hyn-pygm-R	GCGAGGGGTGATACCATTTG		
Hyn-slc2a4-F	AGGAAACCACACTCACAAC	55	236
Hyn-slc2a4-R	AAGACCACAGAACAAACCAA		
Hyn- β -actin-F	TATCCTATTGAGCAGGTATTG	57	144
Hyn- β -actin-R	CCTGTTGGCTTTGGGATTTC		

3. Results

3.1. Transcriptome Sequencing and Differential Expression Analysis

RNA-Seq was performed on orbital fat from fish representing extreme growth phenotypes at two ages: 6 months (M6) and 18 months (M18). Three biological replicates were analyzed for each of the four phenotypic groups (M6B, M6S, M18B, M18S), resulting in

a total of 12 samples. A total of 80.50 Gb of clean data was obtained, averaging 6.71 Gb per sample. The Q30 scores for all samples exceeded 91.73%, and GC content ranged from 46.04% to 51.65% (Table A1). All sequencing data have been deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI; accession number: PRJNA1396904). Principal component analysis (PCA) was performed to assess the overall relationships among samples and the reproducibility of biological replicates. At 6 months, the M6B and M6S groups were clearly separated along the first principal component (PC1), which explained 33.6% of the total variance, and biological replicates within each group clustered together (Figure A1a). Similarly, at 18 months, distinct separation between the M18B and M18S groups was observed along PC1 (36.4% variance explained), with replicates clustering within each phenotype (Figure A1b). These results indicate that the extreme growth phenotypes correspond to distinct transcriptional profiles in orbital fat and that the biological replicates are reproducible.

When comparing the low-body-weight (S) group to the high-body-weight (B) group within the same age cohort, distinct sets of differentially expressed genes (DEGs) were identified. In the 6-month-old cohort, the comparison M6S vs. M6B revealed 54 DEGs, with 10 down-regulated and 44 up-regulated in the M6S group (Figure 1A). In the 18-month-old cohort, the comparison M18S vs. M18B identified 241 DEGs, comprising 45 down-regulated and 196 up-regulated genes in the M18S group (Figure 1B). The DEGs identified in juveniles and market-size fish were remarkably distinct. Despite being associated with the same phenotypic extreme (slow or fast growth), the DEG sets were entirely non-overlapping between juveniles and adults (Figure 2). This indicates that growth divergence is associated with stage-specific transcriptional programs, which likely modulate shared core physiological processes through distinct molecular effectors.

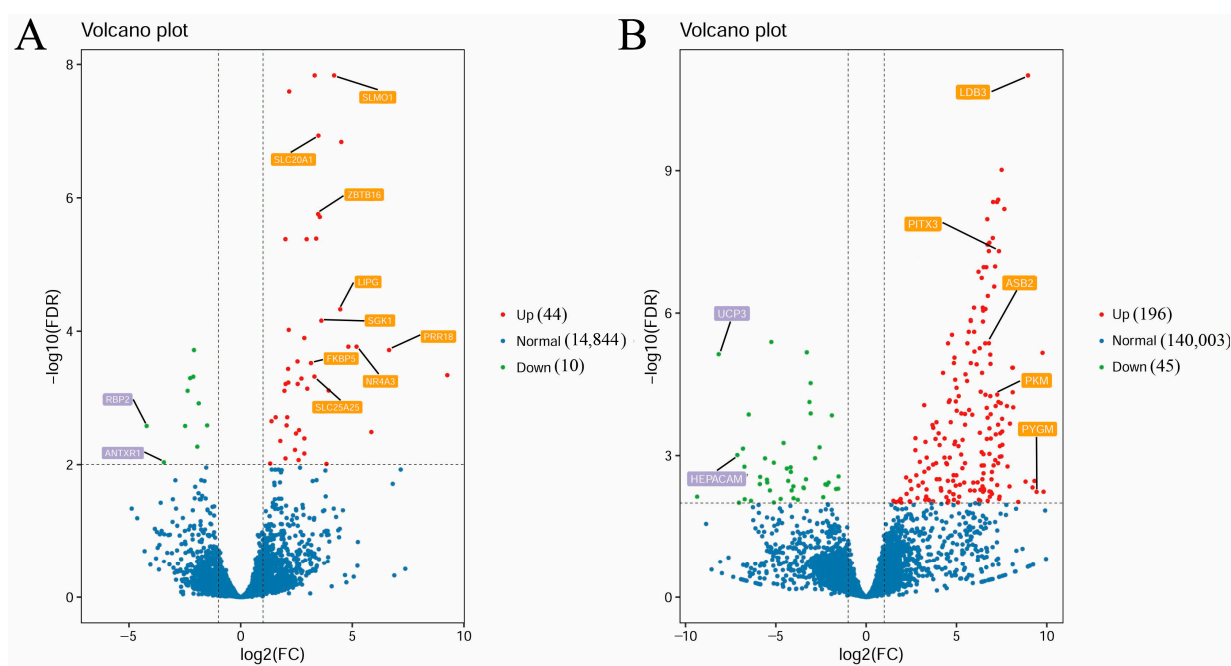


Figure 1. Volcano plots of differentially expressed genes (DEGs) in the orbital fat of bighead carp with extreme growth phenotypes at two ages. **(A)** DEGs between slow-growth (M6S) and fast-growth (M6B) groups at 6 months of age. **(B)** DEGs between slow-growth (M18S) and fast-growth (M18B) groups at 18 months of age. In both plots, significantly down-regulated genes in the slow-growth group (S vs. B) are shown in green, and up-regulated genes are shown in red. Blue dots represent non-significant genes.

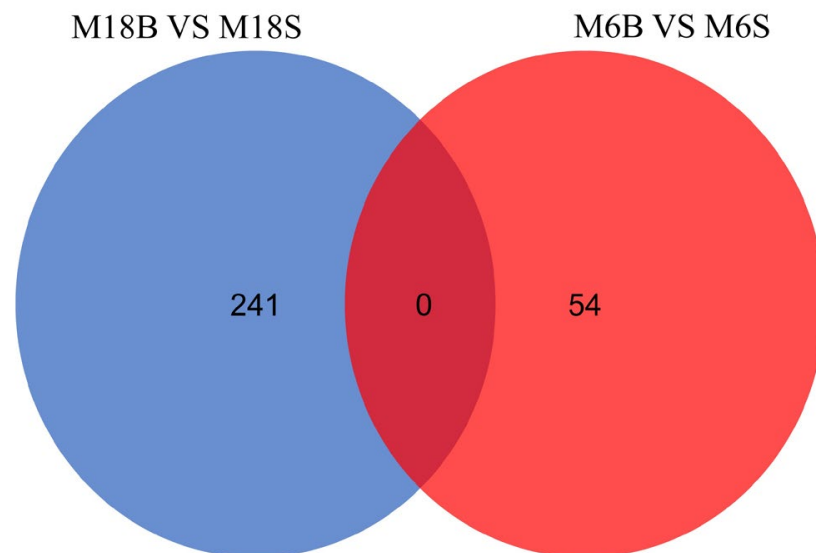


Figure 2. Venn diagram showing the number of differentially expressed genes (DEGs) identified from pairwise comparisons of low-body-weight (S) vs. high-body-weight (B) individuals at 6 months (red circle) and 18 months (blue circle). The absence of overlapping DEGs indicates that growth divergence is associated with distinct, stage-specific transcriptional programs in orbital fat.

3.2. Analysis of GO Functional Categories and KEGG Pathway Enrichment for Differentially Expressed Genes

To elucidate the molecular mechanisms underlying the differential orbital fat size in extreme phenotypes, we performed Gene Ontology (GO) functional enrichment analysis of differentially expressed genes (DEGs) at two critical developmental stages: 6 months and 18 months of age. Distinct yet functionally relevant enrichment patterns were observed across the three GO categories (biological process, BP; cellular component, CC; molecular function, MF) between the two ages, reflecting stage-specific regulatory networks associated with orbital fat development.

In the 6-month-old, the DEGs were predominantly enriched for terms related to central energy metabolism and its regulation (Figure 3). Key biological processes included the pyruvate metabolic process and the generation of precursor metabolites and energy (Figure 3A), while molecular functions featured enzymatic activities critical for carbohydrate metabolism, such as phosphofructokinase activity, fructose-2,6-bisphosphate 2-phosphatase activity (Figure 3B). This pattern suggests that during the juvenile rapid-growth phase, divergent growth is linked to fundamental differences in the capacity for energy production and anabolic precursor synthesis within the adipose tissue. In stark contrast, the transcriptional signature in the 18-month-old cohort had shifted entirely. The most significantly enriched terms were overwhelmingly associated with muscle structure, development, and contractile function (Figure 4). This included biological processes like muscle contraction and myofibril assembly (Figure 4A), localized to cellular components such as the myofibril and sarcomere (Figure 4B), and supported by molecular functions including actin binding and structural constituent of muscle (Figure 4C).

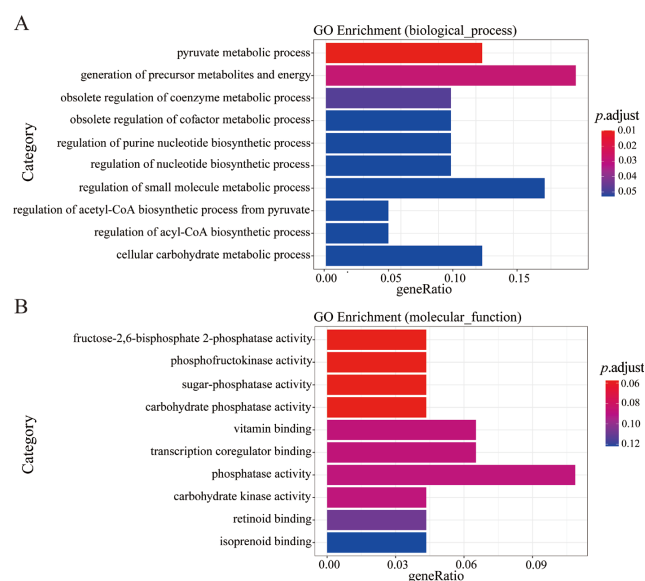


Figure 3. Gene Ontology (GO) enrichment analysis of DEGs in orbital fat from bighead carp with extreme growth phenotypes at 6 months post-hatching. Significantly enriched terms in the (A) biological process (BP) and (B) molecular function (MF) categories are shown. The gene ratio (x -axis) represents the proportion of DEGs mapped to a specific GO term relative to the total number of DEGs. The color intensity of the dots corresponds to the adjusted p -value, indicating the statistical significance of the enrichment.

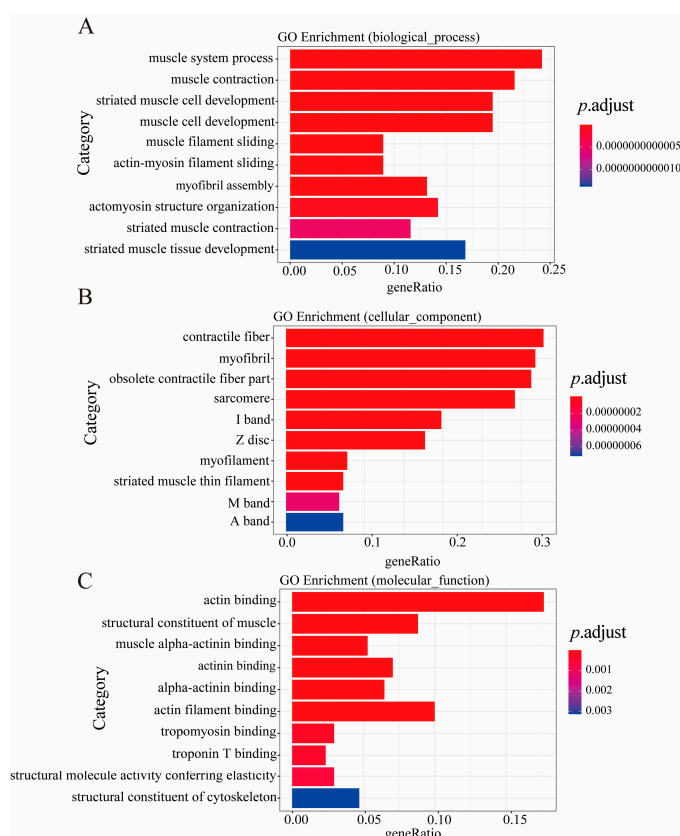


Figure 4. Gene Ontology (GO) enrichment analysis of DEGs in orbital fat from bighead carp with extreme growth phenotypes at 18 months post-hatching. Significantly enriched terms in the (A) biological process (BP), (B) cellular component (CC), and (C) molecular function (MF) categories are shown. The gene ratio (x -axis) represents the proportion of DEGs mapped to a specific GO term relative to the total number of DEGs. The color intensity of the dots corresponds to the adjusted p -value, indicating the statistical significance of the enrichment.

KEGG pathway analysis of the 18-month DEGs identified several significantly enriched pathways (Figure 5). These pathways fell into two broad clusters: one encompassing terms such as Cardiac muscle contraction, Hypertrophic cardiomyopathy, Dilated cardiomyopathy, and Adrenergic signaling in cardiomyocytes; and the other comprising core metabolic pathways, namely Carbon metabolism, Glycolysis/Gluconeogenesis, and Biosynthesis of amino acids. No KEGG pathways were significantly enriched for the 6-month DEGs at the applied statistical threshold.

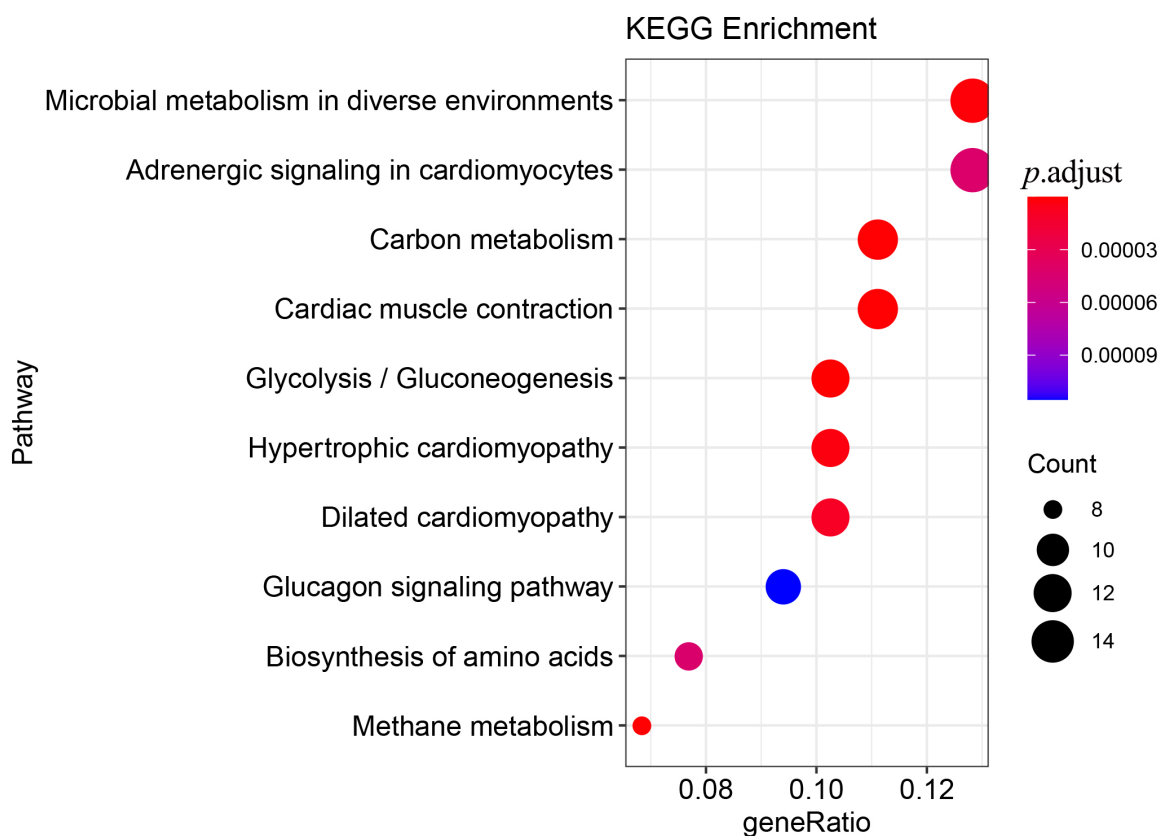


Figure 5. KEGG pathway enrichment analysis of DEGs in orbital fat from bighead carp with extreme growth phenotypes at 18 months post-hatching. The bubble plot displays the top 10 significantly enriched KEGG pathways. The *x*-axis (gene ratio) represents the proportion of DEGs mapped to a specific pathway. The size of the bubbles corresponds to the number of DEGs enriched in the pathway, and the color intensity corresponds to the adjusted *p*-value, indicating the statistical significance of the enrichment.

3.3. Expression Patterns of Candidate Genes in Orbital Fat of Bighead Carp Across Two Developmental Stages

The heatmap (Figure 6) displays the expression patterns of genes that were differentially expressed between growth phenotypes. Further analysis indicated that these genes are associated with key biological processes in orbital fat, including growth regulation and lipid metabolism. Notable differences in gene expression were observed. In the 6-month-old juveniles, several genes involved in stress response and metabolic regulation showed marked expression changes. For example, genes such as serum/glucocorticoid-regulated kinase 1 (*sgk1*) and FKBP prolyl isomerase 5 (*fkbp5*) were upregulated in the M6S group. In contrast, corticotropin-releasing hormone binding protein (*crhbp*) and retinol binding protein 2 (*rbbp2*) displayed higher expression levels in the M6B group (Figure 6A).

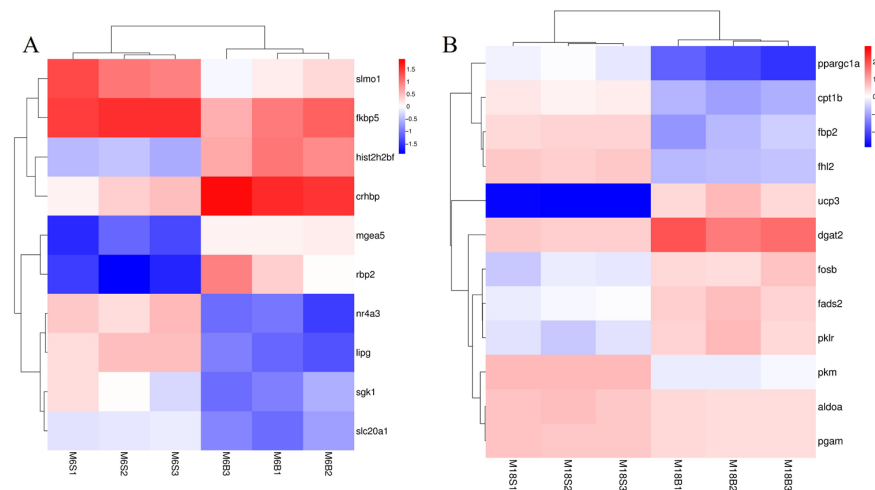


Figure 6. Expression heatmaps of key DEGs in the orbital fat of bighead carp at 6 and 18 months. Heatmaps display the expression patterns of selected differentially expressed genes (DEGs) between small-body (S) and large-body (B) individuals at (A) 6 months and (B) 18 months of age. Expression values are row-scaled Z-scores of FPKM values, with red indicating relatively high expression and blue indicating relatively low expression across samples.

At 18 months, expression divergence between the M18S and M18B groups was also evident. Key genes implicated in lipid synthesis and storage, such as diacylglycerol O-acyltransferase 2 (*dgat2*), were upregulated in the M18B group. Conversely, genes associated with lipid oxidation, including *carnitine palmitoyltransferase 1b* (*cpt1b*), were upregulated in the M18S group (Figure 6B).

3.4. Validation of RNA-Seq Results by qPCR

To validate the RNA-seq results, sixteen DEGs (eight from each developmental stage) were selected for qRT-PCR analysis based on their statistical significance and functional relevance (Figure 7). The expression trends measured by qRT-PCR were highly consistent with the RNA-seq data.

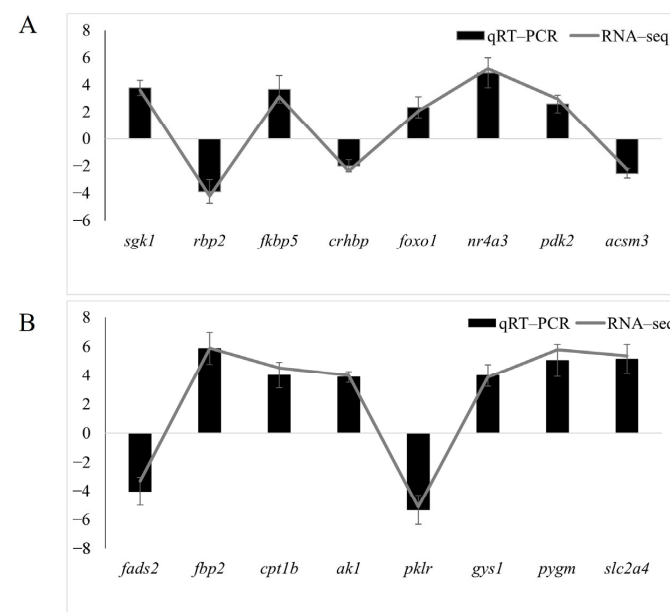


Figure 7. Confirmation of RNA-seq findings by qRT-PCR in orbital fat across two developmental time points. (A) 6-month-old juveniles. (B) 18-month-old market-size fish. Values are presented as mean relative expression $\pm$ SEM ($n = 3$ biological replicates), with β -actin used for normalization.

4. Discussion

While adipose tissue is a key metabolic and endocrine organ, its depot-specific roles in modulating growth variation in fish remain underexplored. This study characterized the transcriptomes of orbital fat from bighead carp exhibiting extreme growth phenotypes at juvenile (6-month) and market-size (18-month) stages. The results indicate that this depot undergoes extensive, stage-dependent transcriptional remodeling, the patterns of which strongly associate with overall growth performance, suggesting a functionally important and dynamic role in somatic growth.

In 6-month-old juveniles, GO enrichment analysis revealed a significant focus on central energy metabolism (e.g., “pyruvate metabolic process”, “phosphofructokinase activity”; Figure 3). This observation suggests that divergent growth at this stage may be associated with differences in the adipose tissue’s capacity for energy production and anabolic precursor synthesis. A prominent feature in M6S individuals was the co-upregulation of *sgk1* and *fkbp5* (Figure 6A), a pattern consistent with activation of the glucocorticoid signaling pathway. *Sgk1* is a primary transcriptional target of the activated glucocorticoid receptor and has been shown to impair insulin/PI3K/Akt anabolic signaling, a central driver of growth [20]. *Fkbp5*, a critical co-chaperone that regulates glucocorticoid receptor sensitivity, is known to be induced by chronic stress and is considered a molecular marker for maladaptive stress responses [21]. This mechanistic interplay is conserved in teleosts, where cortisol signaling and its target genes are integral to a physiological stress response that can suppress growth [22]. This putative stress signature was further supported by upregulation of *nr4a3* (nuclear receptor subfamily 4 group A member 3), a transcription factor implicated in catabolic reprogramming [23]. The co-expression of these genes raises the possibility that the orbital fat of M6S may experience a state of chronic, cell-autonomous stress. In teleosts, such a state, if driven by persistent glucocorticoid signaling, has been proposed to suppress growth performance by promoting proteolysis and lipolysis and by interfering with the anabolic functions of the growth hormone/insulin-like growth factor axis [24]. This transcriptional signature provides a molecular foundation for hypothesizing a link between stress-axis activity and growth variation in juvenile bighead carp. It is also important to note that stress-responsive genes such as *sgk1* and *fkbp5* participate in multiple physiological processes beyond growth regulation, and their expression should be interpreted with this pleiotropy in mind. Future studies incorporating direct physiological measurements (e.g., plasma cortisol, tissue cytokine levels) will be essential to validate and extend these findings.

This stress signature co-occurred with altered expression of lipid metabolism genes, including upregulation of *lipg* (endothelial lipase). In metabolic tissues, chronic low-grade inflammation is a recognized consequence of cellular stress and nutrient imbalance, leading to local insulin resistance and impaired anabolic signaling [25]. This concept is supported by studies in other teleosts; for example, in grass carp, a high-carbohydrate diet induces inflammation, oxidative stress, and metabolic disorders in adipose tissue, ultimately impairing growth performance [26]. Wei et al. [26] further demonstrated that promoting healthy adipose expansion (hyperplasia) could ameliorate these dysfunctions and restore growth. These observations suggest that the M6S transcriptional profile is consistent with a dual burden of glucocorticoid-mediated stress and metabolic stress. While direct evidence for inflammation—such as cytokine profiling or histological assessment—remains to be established, this transcriptional signature provides a foundation for future studies investigating the interplay between stress, metabolism, and growth in bighead carp.

In contrast, M6B exhibited elevated expression of genes favoring stress resilience and anabolic support. For instance, upregulation of *crhbp* (corticotropin-releasing hormone binding protein) was observed in the M6B group. CRHBP attenuates activation of the

hypothalamic–pituitary–interrenal (HPI) axis by binding and neutralizing corticotropin-releasing hormone, thereby buffering the neuroendocrine stress response [27]. The elevated expression of *crhbp* may therefore reflect an enhanced capacity to limit stress-axis activation in fast-growing fish. In teleosts, variation in HPI axis reactivity correlates with individual differences in growth and physiological resilience, supporting the view that efficient stress buffering can be growth-permissive [28].

Two additional genes involved in nutrient-sensitive signaling also showed higher expression in M6B fish. *Rbp2* (retinol binding protein 2) belongs to the intracellular lipid-binding protein (iLBP) family, which is central to the transport and metabolic channeling of retinoids (vitamin A derivatives) [29]. In fish, adequate vitamin A status and functional retinoid signaling are essential for normal growth and physiological homeostasis [30], highlighting the potential importance of efficient retinol handling in supporting an anabolic state. Similarly, increased expression of *mgea5* (meningioma expressed antigen 5, O-GlcNAcase) was detected. This enzyme regulates the dynamic removal of O-linked N-acetylglucosamine (O-GlcNAc) modifications from proteins—a key post-translational mechanism that integrates nutrient and stress signals to fine-tune transcriptional and cellular signaling pathways [31]. Although the role of O-GlcNAcylation in fish adipose biology is not fully characterized, its conserved function as a nutrient sensor suggests that elevated *mgea5* expression may reflect a more active or responsive metabolic signaling environment in the orbital fat of M6B. Collectively, the elevated expression of *crhbp*, *rbp2*, and *mgea5* in M6B highlights a coordinated transcriptional signature that favors stress resilience, efficient retinoid signaling, and adaptive metabolic regulation. This integrated profile likely contributes to a tissue milieu that supports sustained growth, offering insight into the molecular basis of growth divergence within a cohort of juvenile bighead carp.

The transcriptomic divergence at 18 months reveals a fundamental metabolic reprogramming in the orbital fat, centered on lipid and glucose metabolism, which strongly correlates with growth phenotype. In M18S individuals, a predominantly catabolic transcriptional signature was observed, characterized by the upregulation of genes involved in fatty acid oxidation and glycolysis relative to the M18B group. A key marker of this state was the significant upregulation of *cpt1b* (carnitine palmitoyltransferase 1b). CPT1B is the rate-limiting enzyme for mitochondrial fatty acid β -oxidation. In fish models, CPT1B is a critical determinant of energy partitioning; its inhibition redirects metabolism toward glucose utilization and protein synthesis, thereby improving growth performance [32]. Thus, the elevated *cpt1b* expression in M18S fish is consistent with a reinforced catabolic state that may divert energy away from growth-supportive anabolic processes. The significant upregulation of *ppargc1a* (encoding PGC-1 α) in M18S individuals further supports this interpretation. PGC-1 α is a master transcriptional coactivator governing mitochondrial biogenesis and oxidative metabolism. In mammalian systems, PGC-1 α activates programs of fatty acid oxidation [33]. In the context of fish adipose tissue, the concurrent upregulation of *ppargc1a* and *cpt1b* suggests a metabolic shift toward lipid mobilization, which may prioritize meeting basal energy demands over somatic growth investment. Concurrently, the marked upregulation of glycolytic genes (e.g., *aldoa*, *pkm*, *pgam*) and *fbp2* (fructose-1,6-bisphosphatase 2) points to active glycolytic and gluconeogenic flux. The co-activation of lipid oxidation and glucose metabolism could indicate a state of metabolic stress or inefficiency, wherein the tissue simultaneously expends energy through multiple catabolic pathways rather than optimizing energy storage.

Conversely, M18B individuals exhibited a transcriptional profile skewed toward lipid synthesis, modification, and storage. A key finding was the pronounced upregulation of *dgat2* (diacylglycerol O-acyltransferase 2), which catalyzes the final step in triglyceride biosynthesis. In teleosts, functional studies confirm *dgat2*'s role in promoting lipid storage;

for example, its knockdown reprograms metabolism toward fatty acid oxidation [34]. Therefore, the co-occurrence of elevated *dgat2* and reduced expression of fatty acid oxidation markers (e.g., *cpt1b*) in M18B fish strongly indicates a metabolic state biased toward lipid anabolism over catabolism. This pro-storage configuration is consistent with and likely supports the efficient somatic growth of this phenotype. Similarly, higher expression of *fads2* (fatty acyl desaturase 2) in M18B fish suggests an enhanced biosynthetic capacity for long-chain polyunsaturated fatty acids (LC-PUFAs) [4], potentially improving the nutritional quality of stored lipids.

This metabolic dichotomy—marked by anabolism in M18B and catabolism in M18S—supports a hypothesis: the orbital fat of fast-growing fish may be maintained in a more homeostatic, energy-replete state. Such a state may enhance the tissue's capacity to fulfill its dual roles: serving as a stable energy reservoir and, given its anatomical location, providing mechanical cushioning and metabolic support to the eye and associated musculature. A metabolically stable, lipid-replete depot (as suggested by the M18B) would be better suited to maintain its volume and structural integrity, potentially offering more consistent support for the eye and associated musculature. In contrast, a catabolically active, energy-mobilizing state (M18S) might correlate with a less stable tissue volume and compromised supportive function. In vertebrates, adipose tissue is recognized as a multifunctional organ whose metabolic state is integral to its overall physiology, including its capacity to provide mechanical cushioning and structural support [33]. Therefore, the metabolically robust, lipid-storing phenotype observed in the orbital fat of M18B fish may not only favor energy reserve but also sustain the tissue's physical integrity and putative supportive role within the orbital cavity [35]. This perspective considers the observed metabolic shifts alongside the enrichment of pathways implicating structural and functional support (Figures 4 and 5), suggesting that orbital fat metabolism is linked to its role in maintaining the local tissue microenvironment. The enrichment of muscle-related GO terms and KEGG pathways in orbital fat at 18 months was unexpected and warrants careful interpretation. Potential explanations include low-level contamination with extraocular muscle during dissection, misannotation of transcripts, or multi-mapping of reads to paralogous loci, a known challenge in teleost genomics due to whole-genome duplication. Alternatively, this signature may reflect the inherent cellular heterogeneity of adipose tissue. Adipose tissue contains a stromal vascular fraction rich in multipotent adipose-derived stem cells (ASCs) that retain myogenic lineage potential [36–38]. This interpretation is consistent with our previous study, which also detected cardiac muscle contraction pathways in orbital fat across developmental stages [18]. Without histological validation, these possibilities cannot be fully resolved, and this remains a limitation of the current study.

The complete absence of overlapping DEGs between juvenile and market-size fish warrants further consideration. Rather than indicating limited functional relevance, this non-overlap likely reflects stage-specific physiological roles of orbital fat. This interpretation is further supported by the expression patterns of genes involved in DHA synthesis. In our previous developmental study [18], *fads2* was significantly upregulated in 18-month fish compared to 6-month fish, indicating an enhanced capacity for DHA biosynthesis during the market-size stage. In the present study, *fads2* was among the genes most strongly upregulated in fast-growing market-size fish (M18B vs. M18S). Together, these observations suggest that enhanced DHA synthetic capacity in orbital fat is associated with both developmental progression and superior growth performance within the same age cohort, potentially supporting the accumulation of DHA-rich lipids that are critical for growth and tissue function. While these findings highlight stage-specific roles of orbital fat in growth, several limitations should be considered. First, the fish used originated from

a mixed-family population without pedigree information; therefore, we cannot exclude the possibility that some transcriptional differences may reflect family-specific genetic backgrounds. Second, our analysis focused exclusively on orbital fat; although this depot is of commercial interest, growth is a systemic trait regulated by multiple tissues (e.g., liver, muscle, endocrine organs). Third, the transcriptomic findings lack validation at the physiological level (e.g., hormone assays, lipid profiling, metabolic flux measurements), and the enrichment of muscle-related pathways could not be resolved by histology. Addressing these aspects in future research through controlled family designs, multi-tissue transcriptomics, and integrated physiological assays will be essential to validate and extend the findings presented here.

In summary, the expression patterns at 18 months delineate a coherent, stage-specific mechanism: superior growth is associated with an orbital fat transcriptional program promoting lipid synthesis, storage, and tissue homeostasis, while slower growth correlates with a catabolic, energy-mobilizing phenotype. These findings underscore that in market-size fish, growth variation is intimately linked to the metabolic programming of specific adipose depots. In bighead carp, where head size and quality are economically critical, optimizing orbital fat metabolism may therefore impact both somatic growth and product value. Collectively, our findings demonstrate stage-specific transcriptional signatures in orbital fat associated with growth divergence in bighead carp, providing a foundation for future investigations into adipose-mediated growth regulation and potential applications in molecular breeding.

5. Conclusions

The results demonstrate stage-specific transcriptional signatures in the orbital fat that are associated with divergent growth rates in bighead carp. During the juvenile stage, growth divergence appears linked to differential expression of genes related to stress response (e.g., *sgk1*, *crhbp*). In market-size adults, variation correlates with a shift in core metabolic genes favoring either lipid storage (*dgat2*) or oxidation (*cpt1b*). The distinct gene sets between stages indicate ontogenetic reprogramming of growth-associated pathways in this tissue. This study provides a molecular perspective on adipose-mediated growth regulation and highlights potential candidate genes for future aquaculture breeding.

Author Contributions: Conceptualization, J.W. and Q.L.; methodology, J.W., Q.L. and H.T.; software, J.W.; validation, J.L. and Z.S.; formal analysis, G.Y.; investigation, J.W. and Z.S.; resources, J.W. and J.T.; data curation, J.L. and H.T.; writing—original draft preparation, J.W. and Q.L.; writing—review and editing, X.G. and J.T.; visualization, J.W. and G.Y.; supervision, J.L., X.G. and J.T.; project administration, X.G. and J.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (32072973), Doctoral Research Start-up Grant of Xinyang Agriculture and Forestry University (203130), Scientific and Technological Project of Henan Province (252102321036), Scientific and Technological Project of Henan Province (252102110075), Natural Science Foundation of Henan (252300420726).

Institutional Review Board Statement: The animal study protocol was approved by the Academic Committee on Scientific Ethics of Xinyang Agricultural and Forestry University (protocol code XYAFU-AE-2019019 and date of approval 30 May 2019).

Informed Consent Statement: Not applicable.

Data Availability Statement: All sequencing data were uploaded to the Sequence Reading Archive (SRA) of the National Center for Biotechnology Information (NCBI; accession number: PRJNA1396904. Transcriptome analysis orbital fat of *Hypophthalmichthys nobilis*).

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

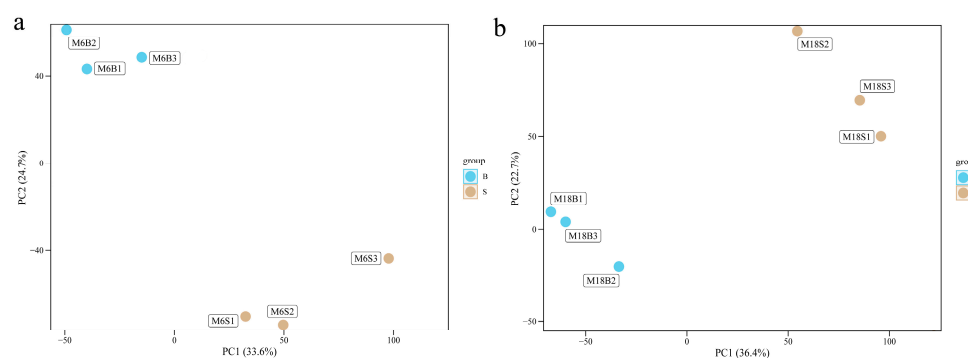


Figure A1. Principal component analysis (PCA) of orbital fat transcriptomes from bighead carp with extreme growth phenotypes at two developmental stages. **(a)** PCA plot of 6-month samples. M6B (fast-growing) samples are shown in blue, and M6S (slow-growing) samples in orange. **(b)** PCA plot of 18-month samples. M18B samples are shown in blue, and M18S samples in orange.

Table A1. The orbital fat transcriptome data quality control results.

Sample ID	Raw Datareads	Clean Datareads	Raw Database	Clean Database	Q20 (%)	Q30 (%)	GC (%)
M6B1	45,856,760	45,751,168	6,871,532,810	6,846,104,587	96.87	91.91	46.97
M6B2	49,951,236	49,905,706	7,485,026,392	7,468,839,798	96.8	91.73	47.73
M6B3	50,993,664	50,708,806	7,616,530,578	7,563,379,410	96.79	91.79	46.35
M6S1	44,931,450	44,870,720	6,719,547,206	6,701,590,018	97.76	93.95	48.13
M6S2	42,405,894	42,349,586	6,347,457,524	6,330,476,364	97.79	93.95	47.31
M6S3	43,683,958	43,618,984	6,537,180,774	6,516,248,839	97.75	93.85	46.04
M18B1	42,915,990	42,639,088	6,431,992,260	6,382,998,824	96.53	91.19	45.37
M18B2	40,726,024	40,701,498	6,100,950,998	6,090,612,188	97.03	92.31	48.38
M18B3	39,462,420	39,435,932	5,914,123,710	5,903,172,099	96.83	91.79	46.6
M18S1	44,018,168	43,960,896	6,559,080,296	6,539,251,144	97.99	94.53	51.65
M18S2	47,170,628	46,951,414	7,060,622,944	7,019,177,020	96.92	92.06	47.89
M18S3	46,093,044	45,579,570	6,901,510,472	6,815,501,103	96.78	91.79	48.58

References

- Tocher, D.R. Metabolism and functions of lipids and fatty acids in teleost fish. *Rev. Fish. Sci.* **2003**, *11*, 107–184. [\[CrossRef\]](#)
- Sheridan, M.A. Lipid dynamics in fish: Aspects of absorption, transorption, deposition and mobilization. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **1988**, *90*, 679–690. [\[CrossRef\]](#) [\[PubMed\]](#)
- Rule, D.C.; Thornton, J.H.; McGilliard, A.D.; Beitz, D.C. Effect of adipose tissue site, animal size, and fasting on lipolysis in bovine adipose tissue in vitro. *Int. J. Biochem.* **1992**, *24*, 789–793. [\[CrossRef\]](#) [\[PubMed\]](#)
- Xie, D.Z.; Chen, C.Y.; Dong, Y.W.; You, C.H.; Wang, S.Q.; Monroig, O.; Tocher, D.R.; Li, Y.Y. Regulation of long-chain polyunsaturated fatty acid biosynthesis in teleost fish. *Prog. Lipid Res.* **2021**, *82*, 101095. [\[CrossRef\]](#)
- Glencross, B.; Carr, I.; Santigosa, E. Distribution, Deposition, and Modelling of Lipid and Long-Chain Polyunsaturated Fatty Acids in Atlantic Salmon Fillets. *Rev. Fish. Sci. Aquac.* **2023**, *31*, 119–140. [\[CrossRef\]](#)
- Tong, J.G.; Sun, X.W. Genetic and genomic analyses for economically important traits and their applications in molecular breeding of cultured fish. *Sci. China Life Sci.* **2015**, *58*, 178–186. [\[CrossRef\]](#)
- Cleveland, B.M.; Gao, G.; Leeds, T.D. Transcriptomic response to selective breeding for fast growth in Rainbow Trout (*Oncorhynchus mykiss*). *Mar. Biotechnol.* **2020**, *22*, 539–550. [\[CrossRef\]](#)
- Wang, Z.; Gerstein, M.; Snyder, M. RNA-Seq: A revolutionary tool for transcriptomics. *Nat. Rev. Genet.* **2009**, *10*, 57–63. [\[CrossRef\]](#)
- Qian, X.; Ba, Y.; Zhuang, Q.F.; Zhong, G.F. RNA-Seq technology and its application in fish transcriptomics. *Omics J. Integr. Biol.* **2014**, *18*, 98–110. [\[CrossRef\]](#)
- Marrero, M.; Monroig, O.; Betancor, M.; Herrera, M.; Pérez, J.A.; Garrido, D.; Galindo, A.; Giráldez, I.; Rodríguez, C. Influence of dietary lipids and environmental salinity on the n-3 long-chain polyunsaturated fatty acids biosynthesis capacity of the marine teleost *Solea senegalensis*. *Mar. Drugs* **2021**, *19*, 254. [\[CrossRef\]](#)

11. Keim, S.A.; Branum, A.M. Dietary intake of polyunsaturated fatty acids and fish among US children 12–60 months of age. *Matern. Child Nutr.* **2015**, *11*, 987–998. [[CrossRef](#)]
12. Sawada, T.; Takahashi, K.; Hatano, M. Molecular-species analysis of fish oil triglyceride by light-scattering mass detector equipped liquid-chromatography. 2. Triglyceride composition of tuna and bonito orbital fats. *Nippon Suisan Gakkaishi* **1993**, *59*, 285–290. [[CrossRef](#)]
13. Hong, H.; Zhou, Y.D.; Wu, H.; Luo, Y.K.; She, H.X. Lipid content and fatty acid profile of muscle, brain and eyes of seven freshwater fish: A comparative study. *J. Am. Oil Chem. Soc.* **2014**, *91*, 795–804. [[CrossRef](#)]
14. Wang, X.Z.; Li, J.B.; He, S.P. Molecular evidence for the monophyly of East Asian groups of Cyprinidae (Teleostei: Cypriniformes) derived from the nuclear recombination activating gene 2 sequences. *Mol. Phylogenet. Evol.* **2007**, *42*, 157–170. [[CrossRef](#)] [[PubMed](#)]
15. Zhang, Z.; Shi, Y.X.; Zhang, J.W.; Liu, Q.G. Experimental observation on the effects of bighead carp (*Hypophthalmichthys nobilis*) on the plankton and water quality in ponds. *Environ. Sci. Pollut. Res.* **2022**, *29*, 56658–56675. [[CrossRef](#)]
16. Wan, P.; Liu, J.; Chen, D.W. Analysis of aroma-active compounds in bighead carp head soup and their influence on umami of a model soup. *Microchem. J.* **2021**, *168*, 106436. [[CrossRef](#)]
17. Zhang, J.; Tao, N.P.; Zhao, Y.L.; Wang, X.C.; Wang, M.F. Comparison of the fatty acid and triglyceride profiles of big eye tuna (*Thunnus obesus*), Atlantic salmon (*Salmo salar*) and bighead carp (*Aristichthys nobilis*) heads. *Molecules* **2019**, *24*, 3983. [[CrossRef](#)]
18. Wang, J.R.; Lei, Q.; Liu, J.; Sun, Z.R.; Yu, X.M.; Guo, X.S.; Tong, J.G. Transcriptome profiling reveals stage-specific regulation of lipid metabolism in orbital fat of bighead carp (*Hypophthalmichthys nobilis*). *Animals* **2025**, *15*, 2602. [[CrossRef](#)]
19. Harshitha, R.; Arunraj, D.R. Real-time quantitative PCR: A tool for absolute and relative quantification. *Biochem. Mol. Biol. Educ.* **2021**, *49*, 800–812. [[CrossRef](#)]
20. Lang, F.; Böhmer, C.; Palmada, M.; Seeböhm, G.; Strutz-Seeböhm, N.; Vallon, V. (Patho)physiological significance of the serum- and glucocorticoid-inducible kinase isoforms. *Physiol. Rev.* **2006**, *86*, 151–1178. [[CrossRef](#)]
21. Binder, E.B. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. *Psychoneuroendocrinology* **2009**, *34*, S186–S195. [[CrossRef](#)]
22. Aluru, N.; Vijayan, M.M. Stress transcriptomics in fish: A role for genomic cortisol signaling. *Gen. Comp. Endocrinol.* **2009**, *164*, 142–150. [[CrossRef](#)] [[PubMed](#)]
23. Maxwell, M.A.; Muscat, G.E.O. The NR4A subgroup: Immediate early response genes with pleiotropic physiological roles. *Nucl. Recept. Signal.* **2006**, *4*, nrs.04002. [[CrossRef](#)] [[PubMed](#)]
24. Mommsen, T.P.; Vijayan, M.M.; Moon, T.W. Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation. *Rev. Fish Biol. Fish.* **1999**, *9*, 211–268. [[CrossRef](#)]
25. Hotamisligil, G.S. Inflammation and metabolic disorders. *Nature* **2006**, *444*, 860–867. [[CrossRef](#)]
26. Wei, M.K.; Tian, Z.Q.; Zhang, N.K.; Zhou, L.; Li, H.D.; Ji, H.; He, G.; Zhang, J.L.; Sun, J. Promoting adipocyte hyperplasia ameliorates high-carbohydrate diet-induced inflammation, oxidative stress, and metabolic disorders and enhances growth performance and immune function in grass carp (*Ctenopharyngodon idellus*). *Fish Physiol. Biochem.* **2025**, *51*, 51. [[CrossRef](#)]
27. Westphal, N.J.; Seasholtz, A.F. CRH-BP: The regulation and function of a phylogenetically conserved binding protein. *Front. Biosci.* **2006**, *11*, 1878–1891. [[CrossRef](#)]
28. Sadoul, B.; Geffroy, B. Measuring cortisol, the major stress hormone in fishes. *J. Fish Biol.* **2019**, *94*, 540–555. [[CrossRef](#)]
29. Parmar, M.B.; Shams, R.; Wright, J.M. Genomic organization and transcription of the medaka and zebrafish cellular retinol-binding protein (rbp) genes. *Mar. Genom.* **2013**, *11*, 1–10. [[CrossRef](#)]
30. Hernandez, L.H.; Hardy, R.W. Vitamin A functions and requirements in fish. *Aquac. Res.* **2020**, *51*, 3061–3071. [[CrossRef](#)]
31. Yang, X.Y.; Qian, K.V. Protein O-GlcNAcylation: Emerging mechanisms and functions. *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 452–465. [[CrossRef](#)]
32. Li, L.Y.; Li, J.M.; Ning, L.J.; Lu, D.L.; Luo, Y.; Ma, Q.; Limbu, S.M.; Li, D.L.; Chen, L.Q.; Lodhi, I.J.; et al. Mitochondrial fatty acid β -oxidation inhibition promotes glucose utilization and protein deposition through energy homeostasis remodeling in fish. *J. Nutr.* **2020**, *150*, 2322–2335. [[CrossRef](#)]
33. Amodeo, V.; Davies, T.; Martinez-Segura, A.; Clements, M.P.; Ragdale, H.S.; Bailey, A.; Dos Santos, M.S.; MacRae, J.I.; Mokochinski, J.; Kramer, H. Diet suppresses glioblastoma initiation in mice by maintaining quiescence of mutation-bearing neural stem cells. *Dev. Cell* **2023**, *58*, 836–846. [[CrossRef](#)]
34. Wang, W.; Tao, W.J.; Hua, J.X.; Lu, S.Q.; Dong, Y.L.; Qiang, J.; Tao, Y.F. MiR-10c targets dgat2 and affects the expression of genes involved in fatty acid and triglyceride metabolism in *Oreochromis niloticus* under heat stress. *Int. J. Mol. Sci.* **2025**, *26*, 9717. [[CrossRef](#)]
35. Cinti, S. The adipose organ: Morphological perspectives of adipose tissues. *Proc. Nutr. Soc.* **2001**, *60*, 319–328. [[CrossRef](#)]

36. Bourin, P.; Bunnell, B.A.; Casteilla, L.; Dominici, M.; Katz, A.J.; March, K.L.; Redl, H.; Rubin, J.P.; Yoshimura, K.; Gimble, J.M. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: A joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy* **2013**, *15*, 641–648.
37. Cawthorn, W.P.; Scheller, E.L.; MacDougald, O.A. Adipose tissue stem cells meet preadipocyte commitment: Going back to the future. *J. Lipid Res.* **2012**, *53*, 227–246. [[CrossRef](#)]
38. Salmerón, C. Adipogenesis in fish. *J. Exp. Biol.* **2018**, *221*, jeb161588. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.