Case Report

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Saltwater Barrel Physiological Compensatory Mechanisms in Hypothermia

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Abstract

The freshwater drum, or Aplodinotus grunniens, is a common type of eurythermal freshwater fish in North America. New aquaculture opportunities emerged as a result of our team's significant advancements in artificial breeding and cultivation in 2019 and research into the physiological responses of the organisms to their surroundings. Its capacity to adapt to hypothermia and maintain metabolic homeostasis, on the other hand, is poorly understood. This experiment used cold stress for eight days at temperatures of 18 °C (LT18) and 10 °C (LT10), with a control temperature of 25 °C (Con), in order to investigate the effects of short-term hypothermia on the physiology and metabolism of freshwater drum. LT18 and LT10 had significantly lower levels of free essential amino acids than Con after two days of cold stress. At LT10, both the activity of lipase (LPS) and the amount of total triglyceride (TG) in the plasma decreased over the course of two days. According to RNA-seq in the liver, metabolic-related signaling, particularly amino acid synthesis and lipid metabolism, was prevented by hypothermia. The PPAR signaling pathway is specifically connected to hypothermia-induced inhibition of lipid and amino acid metabolism. These findings confirmed that PPAR signaling maintains lipid and amino acid metabolic homeostasis under cold stress. These results theoretically support hypothermia resistance in the metabolic homeostasis of freshwater drums.

Keywords: Hypothermia; Lipid Metabolism; Amino Acid Metabolism; Ppar Signaling

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Introduction

Because they are ectotherms, fish's feeding, growth, development, immunity, and reproduction are all affected by changes in temperature. Each species has its own ideal temperature for fish. Temperature extremes, whether extreme low or extreme high, will have some impact on biochemical metabolism and physiological function. Aquatic animals can experience physiological dysfunction and even die from cold stress, a severe form of stress [1].

Temperature and fish metabolism and digestion may be linked, according to studies on fish. Fish tissues' glycogen catabolism accelerates at low temperatures to meet the rising energy demands of cold stress resistance. Meanwhile, cold stress causes blood levels of cortisol to rise. Plasma glucose concentrations significantly rise as a result of gluconeogenesis [2]. Plasma glucose, on the other hand, will gradually fall below normal levels as the cold stress continues. The activity of biological enzymes will also be affected by low-temperature stimulation, which will slow down cellular metabolism and protein synthesis. Low-temperature stress can also encourage fish to synthesize unsaturated fatty acids by increasing the activity of stearoyl-CoA desaturase (SCD) and fatty acid desaturases. Additionally, it may cause cell dysfunction by raising oxidized lipid levels [3].

A group of ligand-activated nuclear receptor transcription factors is known as peroxisome proliferators-activated receptors, or PPARs. PPARs are linked to a number of metabolic-related diseases, including diabetes. They are also important for adipogenesis, lipid metabolism, and maintaining metabolic homeostasis. Additionally, it has been reported that PPAR regulates amino acid metabolism and fatty acid oxidation [4].

Aplodinotus grunniens lives in North America, where it can be found as far south as Mexico and Guatemala and as far north as Canada's Great Lakes region. A. grunniens may be able to adapt to a wide range of water temperatures because of its latitude distribution [5]. Because of its thick back muscles, A. grunniens has a lot of flesh that can be eaten. The flesh is full of protein, amino acids, and fatty acids, making it delicious. These apparent characteristics indicate that the cultivation of A. grunniens has the potential to supply humans with proteins of high quality. However, insufficient resources are still required for the species' domestication, aquaculture, and management methods. Additionally, there has been little scientific investigation into this topic. We introduced American A. grunniens larvae in 2016 for these promising foregrounds, and three years later, we made significant progress in artificial breeding and cultivation. Aquaculture could benefit significantly from this development opportunity. A. grunniens is thought to be able to reproduce at temperatures between 22 and 30 °C [6]. Our previous research revealed that hypothermia disrupted glucose and lipid metabolism and resulted in antioxidant and immune dysfunction in freshwater drums at temperatures below 10 °C [7]. Other early studies indicate that living conditions below 14 °C inhibit its growth, and its minimum survival temperature may be close to 1 °C. However, it is still unknown how hypothermia affects metabolic homeostasis. The control temperature (Con) in this study was 25 °C, with 10 °C (LT10) chosen as the minimum temperature and 18 °C (LT18) chosen as the intermediate temperature based on fish samples from previous experiments. By comparing the variations in plasma biochemical parameters index, fatty acid and amino acid composition, and high-throughput RNA-seq between various groups, researchers were able to investigate the effect of hypothermia on the physiological metabolism of freshwater drum [8]. The role of PPARs in maintaining metabolic homeostasis was also thoroughly investigated. The findings of our study will serve as the foundation for the breeding and production of freshwater drums that are able to withstand extreme temperatures [9].

Materials and Method

Animals for Experiments and Conditions for Raising

The Wuxi Fisheries College at Nanjing Agricultural University served as the setting for the experiment. The laboratory fish were the larvae of the second generation. At the Freshwater Fisheries Research Center of the Chinese Academy of Fisheries Sciences in Wuxi, China, first-generation American parents were shown around. The fish were raised in a system that moved water inside and could be changed to meet the 820 mm requirements of the experiment. For seven days at 25 °C, the fish were fed three to five percent of their body weight of fresh shrimp prior to the experiment [10]. In nine tanks with fifty fish, the freshwater drums were arranged at random. Each treatment had three replicates with water temperatures of 25, 18, and 10 degrees Celsius after acclimatization. Throughout the entirety of the experiment, the temperature of the water was steadily raised by the temperature-adjustable circulating water system to 18 °C and 10 °C within six hours, with dissolved oxygen exceeding 6 mg/L, pH 7.2–7.8, and NH_3 below 0.05 mg/L ***[11]***.

Extraction of RNA

In accordance with the protocols, Total RNA was extracted from the livers of each group using TRIzol Reagent (Takara, Dalian, China). After two days of low-temperature stress, nine liver tissues from each group were subjected to high-throughput sequence. Three fish from the same group, each containing 0.1 g of liver, were randomly mixed among them. The final RNA-seq utilized three biological replicates. After that, our published paper refers to the eukaryotic mRNA enrichment, first and second strand cDNA synthesis, adaptor, and Illumina Hiseq4000 sequencing procedures. There are details about the number of RNAseq reads and the total amount of RNAseq data for each sample [12].

Discussion

For typical growth, fish require relatively constant water temperatures. However, unusual weather frequently causes extreme low or high temperature stress in real-world aquaculture production. The purpose of our investigation is to ascertain how stress caused by low water temperature alters the physiological and metabolic alternation of freshwater drums [13].

The development of fish is characterized by a complex metabolic process that includes the utilization of glucose, amino acids, and fatty acids, the transformation of intracellular proteins, the accumulation of fat, and the regulation of hormones and other nutrients. All of these processes, in addition to the utilization of these nutrients, contribute to the accumulation of lipid and muscle. The process also makes use of hormones and other nutrients. In the low-temperature environment, aquatic animals have developed strategies for overcoming negative stress, such as increasing glycol metabolism and reducing food intake. Tilapia subjected to cold stress experienced an immediate rise in plasma glucose, which decreased two days later. Reduced glucose may also affect the previously reported decrease in immune and antioxidant properties, which may lead to an inadequate energy supply. The fish's health will suffer as a result. In our experiment, the temperature decreased the activity of glucose, lipid, and amino acid metabolic enzymes. According to the findings, a decrease in the activity of digestive enzymes at a low temperature may have an effect on the physiological metabolism of freshwater drums [14].

Protein plays an important role in the nutrition of aquatic animals. Cold stress also affects protein anabolism and decomposition. The activity of biological enzymes is affected by cold stress, which also slows or stops cell metabolism and protein synthesis. According to studies, the composition of free amino acids in various Perccottus glenii tissues altered when subjected to prolonged cold stress. In our study, trypsin significantly decreased under hypothermia stress, indicating a decrease in freshwater drum protein digesting activity. Free essential amino acids (EAA) and free amino acids (FAA) both decreased in quantity. This conclusion is supported by the outcomes of low-temperature stress tests performed on Litopenaeus vannamei.

Additionally, the connection between amino acid metabolism and gene expression was established. Histidine catabolism and folate metabolism are linked by the intermediate metabolic enzyme FTCD. Tryptophan cannot be transported without the presence of AFMID. Tryptophan is broken down by KMO in a significant way. GCDH has the ability to influence the accumulation of glutaric acid as well as 3-hydroxyglutaric acid in the catabolic metabolism of tryptophan, lysine, and hydroxylysine. AFMID, KMO, and GCDH expression decreased under cold stress in this experiment, indicating that freshwater drums' amino acid metabolism was slowed by hypothermia.

PPAR regulates the expression of ACO and CPT1a by accelerating the rate of oxidation of mitochondrial and peroxisomal fatty acids. SCD1, a PPAR target, is the primary enzyme in the biosynthesis of monounsaturated fatty acids. SCD1 was downregulated in our experiment, as was the case in the study on Larimichthys crocea, while ACO, CPT1a, PPAR, and PPAR were upregulated in hypothermia. In oleate-treated macrophages, glycine may also be able to evaluate lipid accumulation and the PPARA/G's lipid-lowering effects as a potential biomarker. Our research demonstrates that activation of the PPAR pathway can also reduce glucose content and inhibit insulin sensitivity by increasing PPAR-related gene expression and lowering glucose levels. This study found that the PPAR pathway maintained the homeostasis of lipid and amino acid metabolism in freshwater drums at low temperatures because the correlation between the PPAR pathway and fat metabolism and amino acid metabolism reversed after cold stresses at 10 °C. Additionally, the connection between PPAR signaling, the metabolism of amino acids, and the metabolism of lipids was established [15].

Conclusion

In this study, catabiosis, lipid and amino acid synthesis, and digestive enzyme activity were all prevented by hypothermia. Analyses of the transcriptome and RT-PCR indicated that lipid and amino acid metabolism was the primary function of DEGs derived from hypothermia. Under low-temperature stress, PPAR signaling was also dynamically linked to the homeostasis of lipid and amino acid metabolism. The metabolic strategies that can be utilized to regulate hypothermia resistance can be used to regulate the molecular basis of hypothermia in freshwater drum.

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