TARGETING THE MILD-HYPOXIA DRIVING FORCE FOR METABOLIC AND MUSCLE TRANSCRIPTIONAL REPROGRAMMING OF GILTHEAD SEA BREAM (*Sparus aurata*) JUVENILES

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SUMMARY

Hypoxia is a common stressor in aquatic environments, and fish reduce feed intake and reorganize its metabolism to limit the tissue O_2 demand. This allows to preserve aerobic metabolism by means of a restricted mitochondrial respiration and a shift in substrate preferences as part of the adaptive response of the skeletal muscle to hypoxia exposure. Herein, we aimed to underline new insights on the mild-hypoxia driving force for metabolic and muscle transcriptional reprogramming of gilthead sea bream juveniles. For this purpose, on-growing juveniles of gilthead sea bream were acclimated for 45 days to mild-hypoxia (M- HYP, 40-60% O_2 saturation), whereas normoxic fish (85-90% O_2 saturation) constituted two different groups depending if they were fed to visual satiety (N; control fish) or pair-fed to M-HYP fish (N-PF). Following the hypoxia conditioning period, all fish were maintained in normoxia and continued to be fed until visual satiation for 3 weeks. The time course of hypoxia-induced changes was assessed by changes in blood metabolic landmarks and muscle transcriptomics before and after exhaustive exercise in a swim tunnel respirometer. Maximumfeed intake was reduced by M-HYP pre-conditioning, and both N-PF and M-HYP experienced an improved feed conversion during the normoxia recovery period. M-HYP conditioning reduced circulating levels of free fatty acids and lactate as part of the hypo- metabolic response to face a reduced O_2 availability. In exercise tests, M-HYP group showeda higher critical swimming speed (U_{crit}) that was preserved along the normoxia recovery period. Changes of circulating metabolites and hormones evidenced an enhanced aerobic ATPproduction in M-HYP fish at the end of the conditioning period, whereas anaerobic metabolism was primed at the end of the normoxia recovery period. Heatmap clustering of muscle differentially expressed (DE) genes, after filtering by ANOVA ($P < 0.05$) and PLS- DA (VIP > 1), grouped together N-PF and M-HYP after the mild-hypoxia conditioning. This yielded 222 differentially expressed genes that were increased up to 421 after exercise exhaustion, although this divergent expression pattern was reduced thereafter to 180 genes at the end of the normoxia recovery period. Gene enrichment analysis and protein interaction plots also highlighted a higher contribution of lipid metabolism and, thus, of aerobic metabolism to whole energy supply, shifting towards a higher anaerobic fitness following normoxia restoration. Despite of these changes in substrate preference, M-HYP fish shared a persistent improvement of swimming performance with a higher critical speed at exercise exhaustion. The machinery of muscle contraction and protein synthesis and breakdown was also largely altered by mild-hypoxia conditioning, contributing this metabolic re-adjustmentto the positive regulation of locomotion and to the catch-up growth response during the normoxia recovery period. Altogether, these results reinforce the large phenotypic plasticityof gilthead sea bream, becoming mild-hypoxia a promising prophylactic measure beforepredictable stressful events.