MECHANO-SENSING IN THE SKELETON OF TELEOSTS: USING MODEL FISH TO UNCOVER THE MECHANISMS

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Introduction

The skeleton is a highly dynamic system that has to combine both toughness and light weight to accomplish its functions. It is commonly assumed that the osteocytes, which are ubiquitous within the bone matrix of tetrapods and in some fish lineages, are the sensors of mechanical stress. They express signaling molecules that coordinate the recruitment and activity of osteoblasts and osteoclasts, essential for the adaptive remodeling of bone mass and structure. Zebrafish and medaka are teleost fish models with cellular and acellular bone, respectively, that has been successfully used to study skeletal development and associated pathologies. The lacuno-canalicular system connecting osteocytes is thought to be responsible for the mechano-transduction of mechanical stimuli in osteocytic bone of tetrapods. While the cellular bone in fish lacks a properly functional canalicular system (Fiaz et al., 2010), it is still able to respond to changes in the biomechanical environment (Cardeira et al., 2015; Kranenbarg et al., 2005). The mechanisms mediating the mechano-sensing/ transduction in teleosts are yet to be determined, with osteoblasts being the strongest candidates as mechanosensors (Fiaz et al., 2010). It has been shown that sustained exercise has positive effects on the skeleton of salmonids, causing a significant increase in mineral content and mechanical strength, while not affecting vertebral bone architecture (Totland et al., 2011, Deschamps et al., 2009). In addition, exercise in fish also improves growth rate, food conversion efficiency, oxygen-carrying capacity, muscle buffering ability and fish metabolism by increasing growth hormone and thyroxine levels while reducing circulating stress hormone (Totland et al., 2011).

Materials and methods

Exposure to exercise

Growing zebrafish (1-2 month old) were submitted for 30 days to two daily periods of 2 hours in sustained exercise using an in-house developed swimming tunnel system generating a water current of 15 cm s^{-1} (exercised fish) or 1 cm s^{-1} (control fish). Fish were fed *ad libitum* twice a day with *Artemia* and artificial diet Zebrafeed (Sparos, Lda). The caudal fins were amputated and allowed to regenerate for 7-9 days while fish were exposed to two daily 2-h periods of increased exercise to analyse the effects on regeneration of bony elements. Expression of bone marker genes was assessed by qPCR and patterns of mineral deposition were determined through micro-computed tomography (microCT), histology and histomorphometry.

Results

MicroCT and histomorphometric analyses showed that changes in bone mass and on the rate of mineral deposition in vertebral bone were induced upon exercise and consequent increase in mechanical load. Alterations in the patterning of regenerating lepidotrichia with a proximalization of the *de novo* formed bifurcations were also observed.

Expression data revealed an overexpression of intermediate osteoblast differentiation markers like *osterix*, while early osteoblastic markers like *bone morphogenetic protein 2* and *runx2* showed a reduction of expression levels.

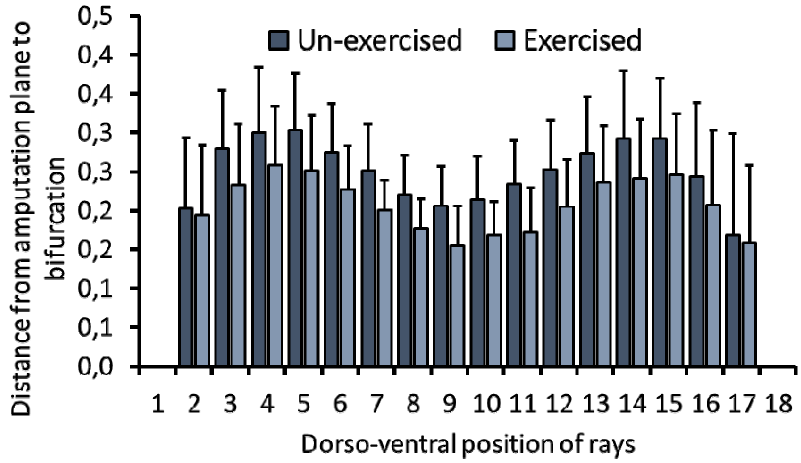

Figure 1. Mean distance until bifurcation in each ray from the dorsal-most to the ventral-most, divided by stump width.

Discussion

This study demonstrated that exercise is able to modulate the formation and remodelling of the skeleton in growing fish and that the expression of genes associated to the differentiation of osteoblasts and maintenance of the extracellular matrix are affected by mechanical loads induced by swimming. The response of osteoblastic cells in acellular bone may lead to metaplastic trans-differentiation into other lineages to better cope with mechanical loads.

The use of model species such as zebrafish will allow the study of cellular and molecular mechanisms underlying the response to mechanical stimuli in a fast and relatively inexpensive manner.

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