ABSTRACT

The pleiotropic hormone, leptin, plays a critical role in energy balance. Studies in mammals suggest that leptin expression should decrease upon cold exposure. We studied the effects of cold shock on leptin expression in koi fish, for which the leptin gene was cloned only 3 years ago. Two groups of koi were exposed to an 8°C drop in water temperature for 30 and 360 minutes and leptin expression was subsequently measured in liver. We used Real-Time quantitative PCR using the ΔΔCt method to measure expression. Leptin mRNA decreased significantly compared to controls at 30 and 360 minutes of exposure. At 360 minutes, expression was greater than it was at 30, but still significantly less than controls. The crash in expression may have a physiologically significant response, although the metabolic disparities between mammals and fish suggest an alternative function. The recovery at 360 minutes suggests rapid acclimation to cold exposure. Cold acclimated carp tend to accumulate fat and preliminary studies show an increase in leptin expression in cold acclimated fish.

INTRODUCTION

Leptin is involved in the regulation of several metabolic processes including body temperature and fat metabolism. Greater than 99% of the 27000 studies on leptin are on mammals, primarily due to the difficulty in cloning other vertebrate sequences. In mammals, cold exposure leads to decreases in leptin expression and circulating titer in serum, although the physiological significance of this response is not known. We tested the effects of cold shock in koi fish, a domesticated form of common carp, by measuring changes in leptin mRNA expression in liver; a major site of expression. Preliminary studies showed that carp, cold acclimated for 6 weeks, have increased hepatic leptin expression. However, it is not known what effects acute cold exposure will have on leptin expression. We hypothesized that cold shock will cause leptin expression to decrease after initial cold exposure, then increase as fish begin to acclimate.

MATERIALS AND METHODS

N = 10 juvenile koi (Cyprinus carpio); 30 total. All fish were kept in 75 gallon recirculating tanks at 20°C. Cold shock fish were removed with fitted net and placed in another identical tank at 12°C for 30 or 360 minutes. Controls were removed from tank then placed back in to account for handling stress. Animals were batch sampled for liver tissue which was preserved in RNAlater® for subsequent RNA extraction. Relative gene expression was measured using RT-qPCR with TaqMan probes and 18S ribosomal subunit as internal control. Data was analyzed by ΔΔCt method (see figure 2); statistical significance (p<0.05) was tested with ANOVA and linear regression.

RESULTS

There was a significant decrease in leptin expression compared to the control at both 30 and 360 minutes (Figure 3). Approx. 2800-fold decrease @ 30 minutes and 680-fold decrease @ 360 minutes. Expression at 360 minutes significantly increased from 30 minutes. Neither batch sampling nor body mass had any effect on expression levels. Expression of 18S also changed significantly (p=0.02 @ 30 min; p=0.002 @ 360 min).

DISCUSSION

The observed decrease in leptin expression implies at least one conserved function of this hormone between fish and mammals. Mammalian studies suggest that a decrease in leptin expression may function to permit increased food intake needed for thermoregulation and non-shivering thermogenesis. The significance of this response is likely to be different in carp since they are ectotherms and curb food intake after cold acclimation.

The significant recovery of expression at 360 over 30 minutes suggests rapid acclimation to cold exposure. This recovery is expected to continue to rise and surpass control levels as the animals shift from carbohydrate to fat-based metabolism. 18S is not an ideal control since it changed significantly. However, we are confident that the change in leptin expression is genuine since the changes in leptin expression were so drastic.

Further investigations are needed to determine if expression levels correlate with protein concentrations. In order to test this, we are developing an ELISA using recombinant carp leptin.

ACKNOWLEDGMENTS

This research was funded by The University of Akron. We would like to thank Tim Astrop, Lee Brucato, Lara Roketenetz, Jacki Schluter, Sara Shaub and Alyssa Stark for their assistance.