

Effect of diet-induced obesity on enteric neurons in zebrafish

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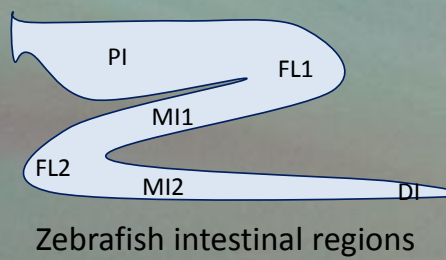


INTRODUCTION

Obesity is a worldwide epidemic and a major risk factor for numerous diseases including cardiac failure, diabetes and cancer. Obesity shows an increasing prevalence demanding for new treatment options and prevention measures. The regulation of feeding behavior and body weight depends on a wide range of neuronal pathways influencing satiety and hunger. Serotonin is one of those players identified to have a profound effect on the energy homeostasis. In the enteric nervous system, serotonin initiates peristalsis and is involved in secretion. At present, biomedical research is predominantly based upon experimental animal models. Recently also zebrafish swam into the spotlights. These fish are characterized by rapid development, easy handling and a high degree of genetic and molecular resemblances for the majority of diseases, including obesity.



Zebrafish after overfeeding (upper) and normal diet (lower)

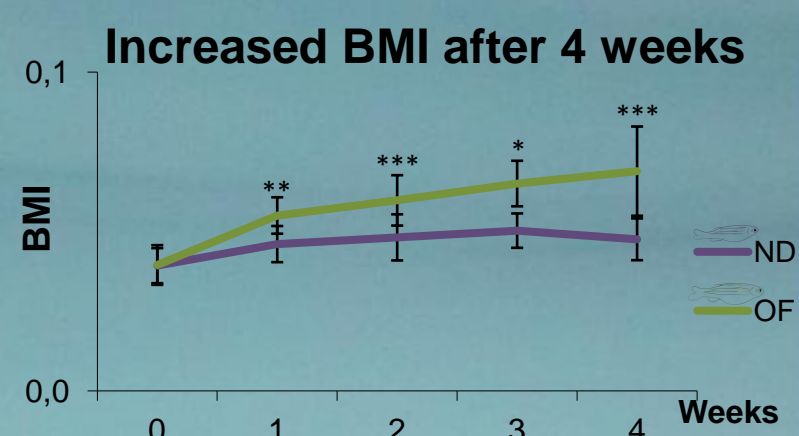
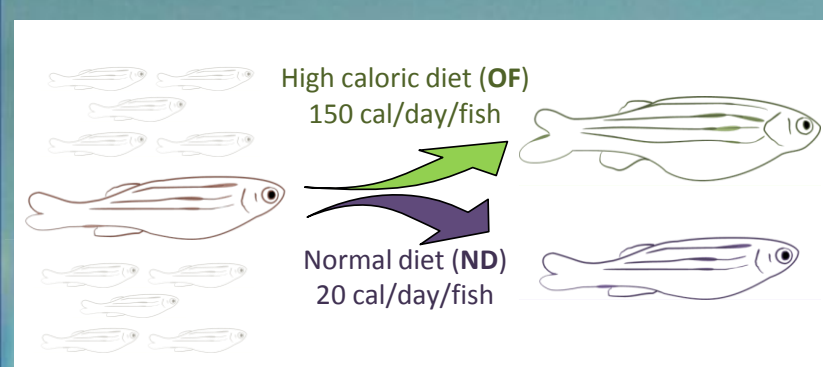


Zebrafish intestinal regions

AIM

The aim of the present study was to create a protocol inducing obesity in adult zebrafish and to investigate the effect of the diet-induced obesity on gastrointestinal (GI) motility and the expression of serotonin in the GI tract.

DIET INDUCED OBESITY



Adult female fish were kept in small colonies and fed either a high caloric diet (overfeeding: **OF**) or a normal diet (**ND**) for 4 weeks. Their body mass index (BMI= g/cm²) was weekly assessed.

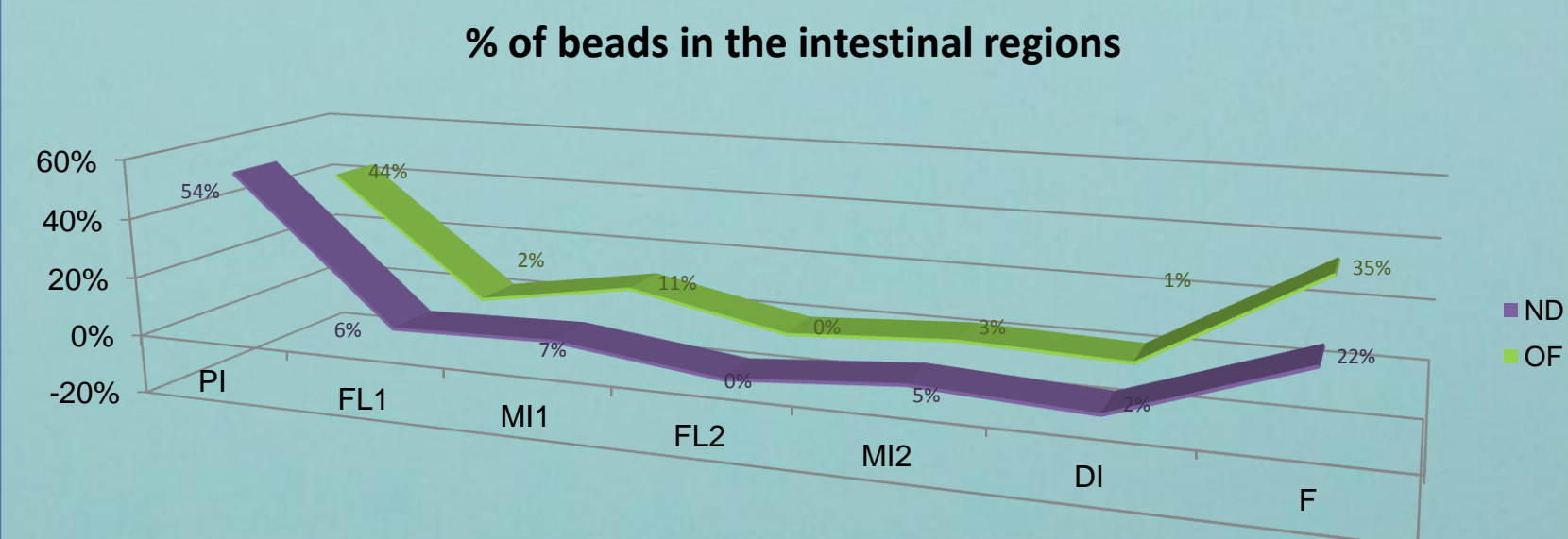
Initially the BMI of OF and ND fish were equal. After 4 weeks overfeeding, the BMI of OF fish increased significantly, but not of ND fish. The BMI was significantly higher in OF fish compared to ND fish during the weekly weighing.

GASTROINTESTINAL TRANSIT

At 4 weeks part of the population (each group n=16) were gavaged 10 glass beads into the proximal intestine to analyse the gastrointestinal (GI) transit. After 6 hours the intestine was dissected out and the glass beads were counted in the different intestinal regions and/or the feces.



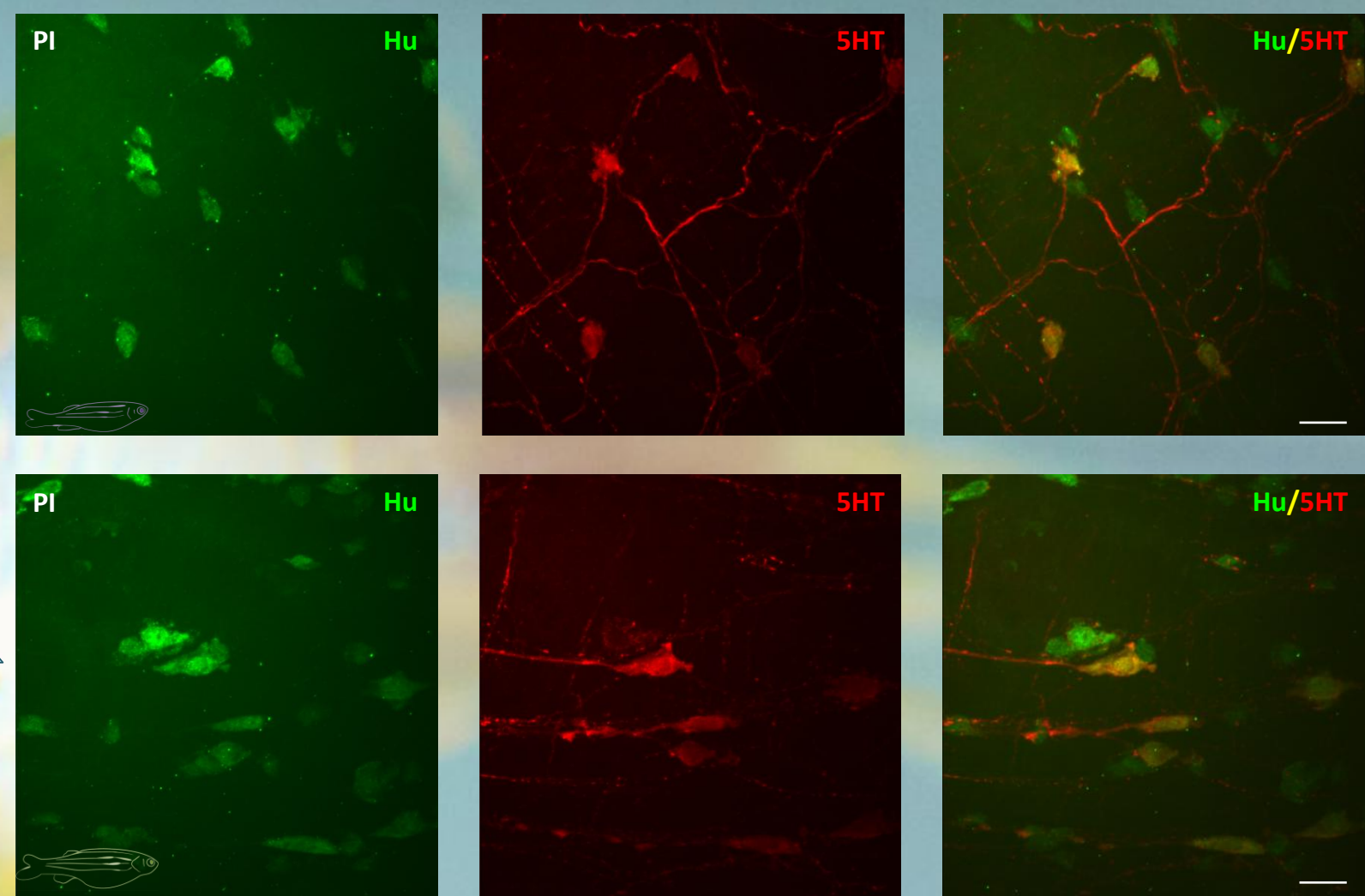
Gavaging zebrafish



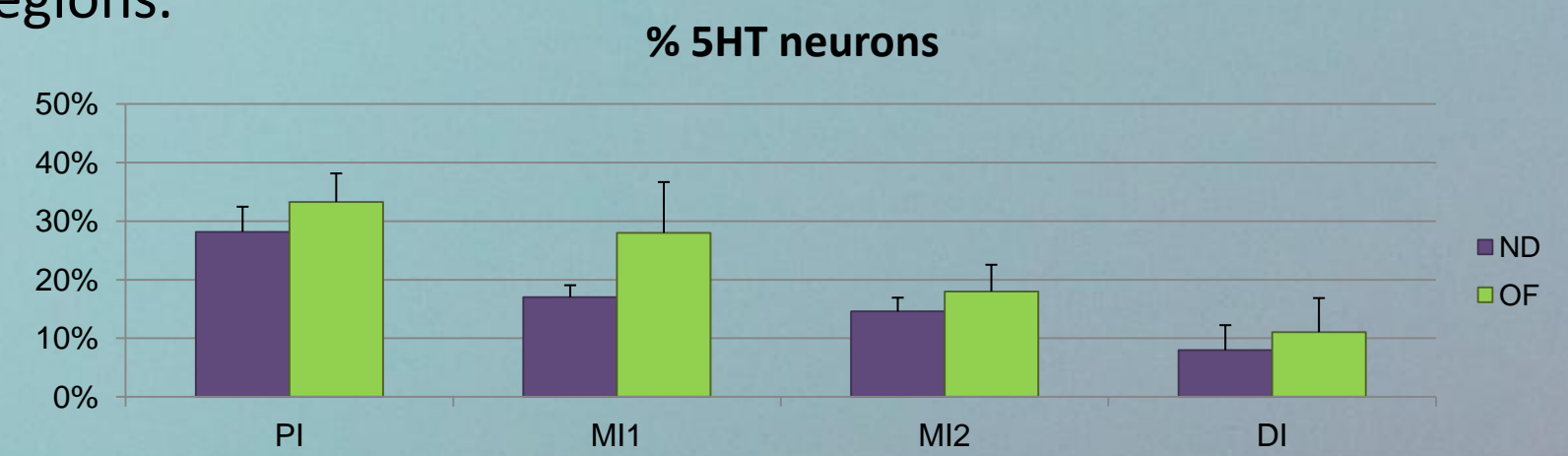
The GI transit did not significantly increase after 4 weeks overfeeding. However, a trend was observed where the beads seem to travel faster down the intestine in OF compared to ND.

SEROTONERGIC NEURONS

The remaining fish (each group n=7) were used to study the expression of serotonin in the enteric nervous system. Multiple immunofluorescence stainings using antibodies raised against the pan-neuronal marker, neuron-specific HuC/HuD proteins, and serotonin were performed on whole mounts.



For the quantitative analysis, serotonergic neurons in the myenteric plexus were counted in the different intestinal regions.



Overfeeding had no significant effect on the proportion of serotonergic neurons in the enteric nervous system. However, a trend was observed after 4 weeks overfeeding in OF fish. In all intestinal regions the proportion of serotonergic neurons was increased in OF fish in comparison to ND fish (overall 23% vs 17%).

CONCLUSION

In this study, we have developed a relatively easy and reliable procedure to generate overfeeding in adult zebrafish. Analysis of the GI tract of obese fish revealed an increased transit and more enteric neurons expressing serotonin. Further studies are required to assess the role of serotonin within the obese zebrafish.

List of abbreviations:

SHT: serotonin; BMI: body mass index; DI: distal intestine; ENS: enteric nervous system; F: feces; FL: flexura; GI: gastrointestinal; IR: immunoreactivity; MI: mid intestine; ND: normal diet; OF: overfeed; PI: proximal intestine

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