1 Abstract

Obesity, a major health problem, is defined by excessive fat accumulation caused by an impaired energy homeostasis. In recent years, the zebrafish (*Danio rerio*) has emerged as an alternative vertebrate model for energy homeostasis and metabolic diseases, including obesity, whereby diet-induced obesity (DIO) in zebrafish shares multiple pathophysiological features with mammalian obesity.

For this thesis a systematic and comprehensive analysis of several pathways of energy expenditure in excessively fed and starved zebrafish was carried out to investigate the impact of caloric intake on scale formation, somatic growth, body weight, fat storage and female reproduction. Middle-aged zebrafish develop severe DIO and excessive feeding results in (I) higher body weight, (II) elevated BMI values, (III) increased amount of triglycerides (TGs) associated with adipocyte hypertrophy and hyperplasia, (IV) increased body length, and (V) enhanced female reproduction associated with heavier ovaries due to accelerated oocyte development compared to caloric restricted fish with an age of six months. Interestingly, juvenile fish are largely resistant to DIO, while BMI and TG values drop in aged fish, pointing to ageing-associated anorexic effects. Furthermore, a differential energy allocation in response to caloric restriction was observed with scale formation prioritised over somatic growth in juvenile zebrafish, while in sexually mature adults, female reproduction is prioritised over somatic growth, and somatic growth over fat storage.

Furthermore, methods were established to quantify food intake and to analyse metabolic rates in juvenile and adult zebrafish. These and other methods were used to study several physiological and behavioural aspects of energy homeostasis in different mutants. Mutation of the *melanocortin receptor 3* or *4* results in increased linear growth and elevated BMI values in excessively fed fish. Of note, neither food intake nor standard metabolic rate were altered in these mutants compared to wild-type zebrafish.

In addition, an ENU-mutagenesis based screen for size of adult zebrafish was performed to identify novel genes involved in energy homeostasis regulation. In total, 29 mutants exhibiting reduced growth were isolated. Analysis of these mutants is ongoing and this thesis provides exemplary information for a selected subset of these mutants.

Finally, systematic analyses of compensatory growth (CG) in juvenile and adult zebrafish and its impact on the etiology of obesity were performed. CG can be induced by excessive feeding following caloric restriction in zebrafish. While induction of CG in juvenile and young adult fish was sufficient to fully compensate for linear growth as well as fat storage, induction in older zebrafish revealed a decreased capability for compensation resulting in partial compensation for fat storage, but complete compensation for linear growth. Interestingly, CG is associated

with an altered fat distribution, most likely with storage in visceral rather than in subcutaneous adipose depots and thereby increasing the risk for development of metabolic morbidities associated with obesity.

Data provided in this thesis will serve as a template for future functional studies to dissect the neuroendocrine regulators of energy homeostasis involved in the etiology of obesity as well as the neuroendocrine and molecular regulation of depot-specific fat deposition. Furthermore, the established methods and presented data could help to elucidate the role of the zebrafish melanocortin system in the regulation of energy homeostasis in regard to similarities and differences between mammals and zebrafish. In addition, mutants isolated in the ENU-mutagenesis based screen could help to identify novel genes involved in regulation of energy homeostasis.