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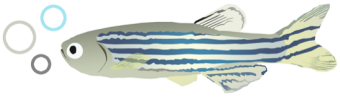

THESIS SUBJECTS ZEBRAFISHLAB

Promoters of these thesis projects are prof. dr. Dries Knapen and/or dr. Lucia Vergauwen.

Theme 1: The role of the paraoxonase gene family in obesity following exposure to environmental pollutants.

Obesity constitutes a major health problem, partly due to the increasing prevalence and secondly because of its associated morbidity. It is associated with increased amounts of adipose tissue as well as fat accumulation in non-adipose tissue such as liver and skeletal muscle. Accumulation of ectopic fat in the liver (non-alcoholic fatty liver disease, NAFLD) is a strong independent marker of dyslipidaemia and insulin resistance predisposing to the development of type 2 diabetes. Besides high caloric diet and lack of physical activity, pesticide exposure and endocrine disruptor pollutants are now also increasingly recognized as an "obesogenic" risk factor. Suspected obesogenic pollutants include polychlorobiphenyls (PCBs) which were widely used in the past (e.g. as coolant fluids) and now still persist in the environment, organochlorine pesticides, phthalates that are added to plastics to increase flexibility and bisphenol A used for the production of plastics. Remarkably, recent genome- and epigenome wide associations studies highlight crosstalk of many obesity-associated genetic variants and environmental factors (diet, pesticides, exercise, alcohol consumption, smoking, drugs, medication) with DNA methylation changes at proximal promoters and enhancers. For example, we recently found a strong association between the paraoxonase 1 (PON1) p.Q192R genotype with pesticide exposure and adverse epigenetic (re)programming of endocrine pathways in obesity and high body fat content. PON members hydrolyze several pesticides, a number of exogenous and endogenous lactones and metabolizes toxic oxidized lipids of low density lipoproteins (LDL) and HDL. A decrease in PON1 expression promotes adverse lipid metabolism and is an important risk factor for cardiometabolic disease and has recently been found to be associated with childhood and adult obesity, liver steatosis and its more severe subtype of steatohepatitis. Differences in PON2 have been associated with obesity susceptibility in brown/white adipose tissue. Given the crucial role of PON members in protecting from adverse environmental exposure and from obesity, there is an urgent need for further molecular and clinical research on (epi)genetic PON(1-3) regulation mechanisms in this area.

In this thesis, a zebrafish model for obesity in humans will be induced by overfeeding using a high-fat diet. This model will be used to study the role of PON(1-3) in the relation between exposure to pollutants, obesity and liver pathology.

Although many different classes of environmental pollutants have been associated with obesity and metabolic disease in general (e.g., PCBs, PBDEs, DDTs, phtalates, organophosphate pesticides, organotins, etc), a direct link between exposure to such compounds and obesity is difficult to establish. Likewise, most studies demonstrate that exposure to these environmental pollutants in zebrafish and many other model species rather is a contributing or aggravating factor for either a pre-existing disease state, or for the onset of metabolic disease triggered by other, more direct factors such as dietary changes. We will therefore investigate the consequences of exposing a zebrafish obesity model to a selection of relevant environmental pollutants.

We will characterize mRNA expression of PON(1-3) in liver under the different experimental conditions. Additionally, we will study mRNA levels of genes involved in lipid and general energy

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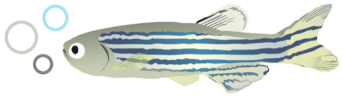


metabolism (e.g., different lipases, TAG synthesis versus carbohydrate metabolism, PPARα/γ, leptin, ghrelin, etc.) in liver and muscle tissue, as well as in carcasses (in zebrafish, adipose tissue is not a single homogeneous tissue but adipocytes are deposited in subcutaneous, intra-abdominal and intramuscular positions. We will also characterize the obesity phenotype: 1) at the organismal level, we will measure the condition factor (a weight/length relation similar to the BMI in humans), oxygen consumption to determine physiological aerobic/anaerobic scope, swimming performance, and reproductive capacity; 2) at the tissue level, we will study the energetic composition of liver and muscle tissue (lipids, proteins, carbohydrates), and use Oil red O neutral lipid staining to quantify lipid accumulation. We will use a combination of different neutral and fluorescent dye techniques to visualize the relevant metabolic tissues (pancreas, liver and adipose tissue) using standard, confocal, and light-sheet illumination confocal microscopy; 3) at the biochemical level, we will study PON(1-3) enzyme activity, quantify blood HDL and LDL/VLDL cholesterol, plasma triglycerides, and measure ETC activity to determine biochemical aerobic scope.

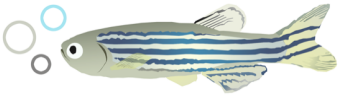
THEME 2.
Toxicology of lipophilic compounds in the zebrafish embryo

At least 60% of the most commonly used chemicals in industrial applications are thought to act by narcosis. In a toxicological context, the term narcosis refers to lipophilic chemicals accumulating in cellular membranes as a function of their degree of lipophilicity, thereby causing toxicity. It is assumed that with increasing lipophilicity, chemicals have an increasing tendency to accumulate in membranes and disrupt their integrity and function. These chemicals can adversely impact a wide range of species, when they enter into the environment. Generally, loss of reaction to external stimuli, loss of equilibrium, decline in respiratory rate and death are expected in fish. However, the mechanistic details of how these chemicals cause toxic effects at the molecular and cellular level are largely unknown. Environmental risk assessment for this large array of chemicals is therefore currently mainly based on standard toxicity tests and lethality estimates using quantitative structure activity relationships (QSARs), which predominantly rely on lipophilicity estimates.

At this point, it is unclear which membrane types and which proteins are functionally impaired by narcotics. We hypothesize that the cell membrane is the first target of lipophilic compounds, which may then further partition into different organelle membranes. Many membrane-bound processes such as the mitochondrial electron transport chain are vital to cell survival and may be affected. The thesis subjects related to this theme will focus on further unravelling the mechanism of narcosis by identifying target membranes and investigating toxicity responses in the zebrafish embryo model at different levels of biological organisation (molecular, biochemical, physiological) and using a variety of techniques.

In this thesis molecular, biochemical and physiological effects of lipophilic chemicals will be characterized in zebrafish embryos.

In this thesis, zebrafish embryos will be exposed to narcotic chemicals. Disruption of cellular processes is often related to changes in transcript levels of related genes. At the molecular level transcriptional expression of selected genes (e.g., components of the electron transport chain) will be analysed using qPCR. At the biochemical level, the effects of narcotics on membrane-bound processes (e.g., mitochondrial electron transport chain activity) will be further explored. Such effects can be further characterized. For example, in cases where aerobic cellular respiration is disrupted, there may be a shift in the balance between aerobic and anaerobic ATP synthesis. By measuring lactate levels and



lactate dehydrogenase (LDH) activity in exposed zebrafish embryos, the relative contribution of anaerobic and aerobic ATP synthesis can be evaluated. Responses will also be investigated at the physiological level which is of direct ecological relevance for risk assessment. Heart rate, swimming behaviour, oxygen consumption, growth and survival will be observed, and related to the findings on the molecular and biochemical level. We will observe loss of equilibrium, perform automated swimming behaviour analysis using a Zebrabox (Viewpoint), measure growth as larval length, monitor survival and determine heart rate in 1 day old embryos. Oxygen consumption measurements will be performed using small respiration chambers with optical oxygen probes suitable for zebrafish embryos. This knowledge is vital to understanding the mechanism and the impact of narcotics on the normal function of organisms.

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