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Abstracts

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Sponsor av NSFTs vintermøte 2023:

Basic & Clinical Pharmacology & Toxicology

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Basic pharmacology

Dissecting the roles of genes and lipids in NAFLD

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Background and Objectives: Nonalcoholic fatty liver disease (NAFLD) encompasses many abnormalities, ranging from accumulation of fat within hepatocytes (steatosis), to fibrotic non-alcoholic steatohepatitis (NASH), cirrhosis, and cancer. It is believed that when the liver is unable to effectively store lipids into stable intracellular lipid droplets (LDs), lipotoxic lipids accumulates in the cytosol and contribute to the development of inflammation and fibrosis. Previous lipidomic studies in humans have identified several lipid species that are associated with progression of a healthy liver to NASH and fibrosis. We have previously utilized Hybrid Mouse Diversity Panel (HMDP) to investigate genetic regulation of a large array of liver lipids contributing to hepatic steatosis and insulin resistance (Norheim et al, Mol Syst Biol, 2021). However, it is unclear how these lipids may contribute to the development of NASH.

Methods: Mice carrying transgenes for human apolipoprotein E*3-Leiden and cholesteryl ester transfer protein and fed a "Western" diet were studied on the genetic backgrounds of over 100 inbred mouse strains. Liver fibrosis were quantified with picrosirius red and ~500 liver lipids were measured using HPLC coupled to TOF-MS

Results: A previous study on this mouse population has shown that the mice developed hepatic inflammation and fibrosis that was highly dependent on genetic background, with vast differences in the degree of fibrosis (Hui et al, Hepatology, 2018). In the present project, we are performing different systems genetics approaches on the same mouse population to identify genes contributing to altered levels of a variety of lipotoxic lipids in livers with different degrees of fibrosis. Our screen will provide a novel and rich resource that can be used to search for causal genes involved in the production and /or accumulation of lipids associated with NASH development. We are in the process of studying the roles of genes and lipids in a detailed NASH progression study performed on mice, as wells as in cultured cells (stellate cells and hepatocytes), and in human liver organoids.

Conclusion: Our overall goal in this project is to identify and molecularly clarify how specific genes and hepatic lipid species contribute to development of NASH.

Hui ST et al: *The Genetic Architecture of Diet-induced Hepatic Fibrosis in mice with a Humanized Lipoprotein Profile.* Hepatology. 2018

Norheim F et al: *Regulation of liver lipids in a mouse model of insulin resistance and hepatic steatosis.* Molecular Systems Biology, 2021

Toxicology

PFAS exposure and health risk in Norway

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Objectives: To estimate dietary exposure to the polyfluorinated alkyl substances PFOS, PFOA, PFHxS and PFNA in Norway and to relate the estimated intakes to the tolerable weekly intake of 4.4 ng/kg bw per week set for the sum of the four PFASs by the European Food Safety Authority (EFSA) in 2020 (EFSA 2020).

Methods: Food consumption data were from the national dietary surveys Spedkost 3 (1-year-olds, conducted in 2019, FFQ), Småbarnskost 3 (2-year-olds, conducted in 2019, FFQ), Ungkost 3 (4, 9-, and 13-year-olds, conducted in 2015, 4 days food diary), and Norkost 3 (Adults, conducted in 2010-2011, two 24-hour-recall interviews).
Concentrations of PFASs in food were obtained from the EFSA database, which includes Norwegian data on fish from the Institute of Marine Research.
Each food item reported consumed in the national dietary surveys was assigned a concentration from similar foods/food group. For food items with no concentration data, the exposure from this food was set at zero. Chronic exposure was estimated by a mixed model based on Bayesian estimation. Population representativeness was obtained by sample-balancing for gender, education, age, and geographic regions.

Results: The mean intakes of the sum of four PFASs ranged from 6.5 to 18 ng/kg bw/week (lower bound, LB). The corresponding 95th-percentile estimates ranged from 8.8 to 35 ng/kg bw/week. There was a high proportion of food samples with concentrations below levels of quantification (LOQ), which were rather high. Large uncertainties were reflected in large difference between LB and upper bound (UB) exposure estimates (e.g., for adults the UB was 18-fold higher). Based on toxicokinetic modelling and available serum concentrations EFSA (2020) reported that the true exposure is more likely to be closer to the LB than the UB estimates.

The highest contribution to the total intake was from PFOS, followed by PFOA, together contributing approximately 80-90% of the sum of the four PFASs.

Fish contributed most (31-42 % for different age groups) to the intake, followed by fruit/vegetables/potatoes (17-32 %) and eggs (13-19 %). Lean fish contributed approximately equally as fatty fish to the total intake from fish.

Conclusion: Current dietary exposure to PFASs in Norway is above the TWI in large parts of the population. This is confirmed by measured serum concentrations in adults. Exposure above the TWI is of health concern.

References:

VKM 2022: Benefit and risk assessment of fish in the Norwegian diet. VKM report 2022:17
EFSA 2020: Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA Journal 2020;18(9):6223

Understanding bone remodelling and bone loss.

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Bones are not inert structures, but change during one's lifetime. This change is referred to as bone remodeling, and involves the resorption of damaged or old bone followed by the deposition of new bone material.

The cells responsible for the resorption are the osteoclasts, derived from monocytes, while the cells responsible for the deposition phases of bone remodeling are the osteoblasts, derived from stem cells of mesenchymal lineage in the bone marrow. The remodeling process is regulated by cross-talk between osteoblasts and osteoclasts through secreted factors and cellular contact, however osteocytes, the most abundant cell type of bone, play an important role in this process by controlling osteoblast and osteoclast differentiation and activity.

Osteoporosis is a common disease characterized by a systemic impairment of bone mass and microarchitecture that results in fragility fractures. The aim of osteoporosis therapy is either to inhibit osteoclast differentiation and/or activity, or to enhance the differentiation of the osteoblast, and traditionally, the effect of a drug is tested on the one cell type it is supposed to affect. This strategy may be too narrow in relation to how complex the regulation of bone mass is. Lack of *in vitro* 3D model systems that mimic bone, with the three main cell types combined, makes it difficult to identify the overall effect, the cross-talk between the cells and cellular and molecular mechanisms triggered, and thus possible side effects.

In a patient, hormonal signals, combination treatment, cellular senescence, inflammation and modifications of the extracellular matrix will affect the activity of the bone cells directly or indirectly, influence not only the remodeling capacity, but also the individual outcome of the chosen treatment strategy.

Summary

Drugs and new pharmacological targets in the treatment of osteoporosis

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In the treatment of osteoporosis, there are mainly two approaches to reduce the progression of the disease. One approach is to reduce bone resorption by inhibiting osteoclasts (bone-resorptive cells), whereas the second approach is to enhance bone formation by stimulating osteoblasts (bone-forming cells). Currently, most drugs approved against osteoporosis are reducing bone resorption, like the bisphosphonates, denosumab, selective oestrogen receptor modulators and hormone replacement therapy. The bisphosphonate alendronate is presently the drug of choice. Over the last years, there has been more focus on developing drugs enhancing bone formation. Teriparatide, an analogue of the parathyroid hormone, stimulates osteoblasts more than osteoclasts, thus favouring bone formation. Sclerostin is a protein secreted by osteocytes (the main cell type in mature bone) and inhibits osteoblast but stimulates osteoclasts. The most recent drug on the market is romosozumab, a monoclonal antibody against sclerostin and favouring bone formation. My research group is studying legumain, a cysteine protease inhibiting osteoblast and favouring adipocyte differentiation of bone mesenchymal stem cells. Our hypothesis is that legumain is a potential pharmacological target in the treatment of osteoporosis by enhancing bone formation using selective legumain inhibitors. The talk will summarize knowledge of the drugs presently used in the treatment of osteoporosis. In addition, potential new drug targets will be discussed.

Stem cell and Sarcoma Single-cell landscapes and beyond

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Sarcomas are rare and aggressive tumours where precise diagnostic and prognostic classification is challenging due to lack of molecular markers. Considerable cancer cell heterogeneity in these tumours have made it difficult to identify definitive disease-associated genes. Liposarcoma (LPS) is the second most common type of sarcoma and can develop from adipose stem cells (ASCs) in the fat tissue anywhere in the body. To decipher the cancer cell heterogeneity, we used an integrated single-cell approach to analyse patient derived LPS cells and compare with ASCs during a time course exposure to an adipose lineage commitment environment. We have identified LPS cell populations with a progenitor phenotype that resist adipose differentiation. Complementary we have mapped the LPS chromatin landscape. Finally, we have stained LPS patient tumour samples and found that these distinct progenitor signatures hold great potential for future therapeutic exploration.

Advancing metabolomics towards the regulatory application of chemical grouping

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Metabolomics has reached a critical point in determining its value to regulatory toxicology. Building on 20 years of research, the first metabolomics study to support chemical grouping/read-across was recently submitted to the European Chemicals Agency, metabolomics best-practices for various applications including grouping were published in *Nature Communications*, and the OECD Omics Reporting Framework has been developed. However, further confidence in this technology needs to be built, including through regulatory-relevant case studies, as well as research to demonstrate its reliability.

Grouping and read-across is frequently used in chemical risk assessment to fill data gaps for human health and environmental endpoints, to minimise vertebrate animal testing and reduce costs to industry. Traditionally, substances are grouped based on structural and/or physico-chemical parameters, leading to grouping hypotheses that are often rejected by the regulator. Confidence in the grouping hypothesis could be improved by adding relevant experimental evidence based on the molecular ('omics) responses to chemical exposure.

This presentation will introduce metabolomics more generally, then describe an application of metabolomics to chemical grouping - conducted in collaboration with ECHA - that investigated grouping based on the molecular responses of *Daphnia magna* to seven disperse azo dyes. Also, the talk will briefly introduce a number of other advances in the application of metabolomics towards regulatory toxicology, including the new MTox700+ list of metabolic biomarkers.

Environmental toxicology

Assessment of bioavailability of engineered nanomaterials by single particle ICP-MS and amphipod haemolymph isolation

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Objectives
Bioaccumulation tests with the benthic freshwater amphipod *Hyalella azteca* (HYBIT) are currently discussed as a part of a tiered approach to determine the bioaccumulation potential of engineered nanomaterials (ENMs). This may allow to avoid further vertebrate tests for regulatory bioaccumulation assessement, such as the fish bioaccumulation tests (OECD TG 305). However, the small size of the amphipod, does not allow to distinguish the ENMs in their intestinal content from the real incorporated, bioavailable fraction (ENM in the tissue or body fluids). Even if a concept of a tiered assessment scheme based on ENM-HYBIT that takes this ambiguity into account with adjusted endpoints exists, methods to gain further data on the bioavailability of ENMs are requiered. Existing mesthods for the localization of tissue-incorporated ENMs (e.g. correlative microscopy or micro X-ray fluorescence imaging) require sophisticated time- or cost-intensive analytical methods, very high exposure and body burden concentrations.

Methods
We coupled a simple, microcapillary based method that can be used in any laboratory to isolate the haemolymph of exposed amphipods with the analytical method of single particle Inductively Coupled Plasma - Mass Spectrometry (spICP-MS). *H. azteca* was exposed to AgENMs and $AgNO_3$ (ionic comparison group) and the isolated haemolymph from 20 animals per replicate was collected, pooled, diluted and analyzed for the presence of AgENMs.

Results
Despite the small sample volume, the method allowed us to measure AgENMs in the haemolymph. The measured AgENMs showed a strong comparability in size to the particles measured in the exposure medium. Ag in particulate form was also found in the haemolymph from the $AgNO_3$ treatment which was of different size and more heterogenous size distribution. This indicates the formation of secondary particles, as has already been described for other aquatic species upon $AgNO_3$ exposure.

Conclusion
Our results show that the proposed coupled methods are suitable to assess the bioavailability and tissue translocation of ENMs. This approach provides valuable insight that can support data interpretation from the ENM-HYBIT to provide a higher degree of certainty for the assessments. Secondary particles (particles generated by precipitation of Ag in the medium or in the organism) as well as pseudoparticles can also be identified by comparison with spICP-MS measurements of the exposure media.

Enhancing the quantitative understanding of an adverse outcome pathway network for mitochondrial dysfunction using *in vitro* and *in silico* new approach methodologies

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Session: Environmental toxicology

Objectives
Mitochondria are the energy warehouses in eukaryotes. A wide range of chemicals can affect mitochondrial energetic functions. Uncoupling of oxidative phosphorylation (OXPHOS) is one of the most common modes of action of mitochondria toxicants leading to adverse effects of regulatory concern (e.g., growth inhibition). By aligning the adverse outcome pathway (AOP) concept and the 3Rs principles, this study aims to use new approach methodologies (NAMs) to support the development of a quantitative AOP network (qAOPN) and a tiered animal alternative testing strategy for cost-efficient hazard assessment of mitochondrial uncouplers. The AOPN linking mitochondrial uncoupling to growth inhibition in eukaryotes is currently under active development (OECD AOP project 1.92), with one of the linear AOPs being endorsed by WPHA/WNT (https://aopwiki.org/aops/263). To quantify key events (KEs) in this AOPN, a suite of high-throughput *in vitro* bioassays was conducted with the zebrafish liver (ZF-L) cell-line.

Methods
ZF-L was exposed for 2, 6, 12, 24, 48 and 72 h to nine concentrations (0.001–100 μM) to the model uncoupler carbonyl cyanide m-chlorophenyl hydrazone (CCCP). The KEs measured include: mitochondrial membrane potential (MMP), ATP level, calcium signalling, cytotoxicity (metabolic activity and cell membrane integrity) and cell proliferation. In addition, chemical analysis was performed of CCCP in the cells and exposure media.

Results
Temporal and concentration dependent responses of ZF-L cells to CCCP were observed for the KEs of interest as early as 2h of exposure. The uptake and metabolism of the different CCCP concentrations corresponded well to the temporal effect response observed in the cells. The most sensitive and responsive (2h) KE was uncoupling of OXPHOS as indicated by decreased MMP. A decrease in ATP was observed after 6 h of exposure, followed by a clear concentration-dependent decrease in cell proliferation. There was no apparent change in cytosolic calcium signalling until 6-12h of exposure. A slight increase in cell death was also observed when exposure duration increased. The data were further used to construct quantitative KE relationships in an AOPN. The coefficients for molecular initiating- and several of the key events were statistically significant in the AOPN.

Conclusion
This study has demonstrated the use of NAMs for qAOPN development. Among the few studies that take temporal responses into account, the current work has supplied novel causal knowledge and a new workflow for qAOPN construction which could be easily adopted by future studies.

Environmental Toxicology

Uncovering metabolic disturbances in chitin metabolism and phenotypical endpoints after exposure to teflubenzuron in *Daphnia magna*

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Objectives
In order to grow, reproduce and survive, arthropods need to shed their cuticle (exoskeleton) periodically through molting. In order to molt, arthropods depend on the formation of a new, larger cuticle stable to support muscular contractions during the molting process. Chitin synthesis is crucial for molting and has been exploited as a target for control of unwanted arthropods by development of chitin synthesis inhibitors (CSIs). We investigated the effects of the chitin synthesis inhibitor teflubenzuron (TEF) on selected metabolites of the chitin biosynthesis – and degradation pathway, as well as on molting and associated mortality. We aimed to identify responsive metabolites in the pathway and find possible metabolic markers to predict adverse phenotypical effects.
Methods
In order to achieve our objectives, we exposed neonatal *Daphnia magna* to TEF (0.1-8 µg/L) for 48h. After 24h of exposure, we sampled *D. magna* for subsequent metabolite extraction and quantitative LC/MS analysis of 6 selected metabolites involved in chitin synthesis- and degradation.
Results
We observed a dose dependent decrease in Trehalose (Tre) and increase in Glucose (Glc) levels, as well as non-monotonic responses of Glucosamine (GlcN) and *N*-Acetylglucosamine (GlcNAc). The observed 24h benchmark dose concentrations (BMCs, based on zSD Benchmark response) for the change in Tre, Glc, and GlcNAc (0.19, 0.53, and 0.44 µg/L, respectively), were found to be lower than both 24h and 48h BMCs for molting (2.64 and 2.63 µg/L, respectively) and survival (3.16 and 0.95 µg/L, respectively), indicating that metabolic modifications occurred prior to and at lower concentrations of TEF than adverse phenotypical (apical) effects. Alterations in levels of metabolites involved in both chitin synthesis and degradation suggest that multiple pathways, that are key to chitin synthesis and ultimately integrity, are affected by exposure to TEF.
Conclusions
In summary, quantified carbohydrates involved in chitin synthesis and degradation were found to be more sensitive endpoints than phenotypical effects and establish themselves earlier than the onset of molting disturbances or mortality.
Acnowledgements
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Decoding marine mammal toxicology through *in vitro* and *in silico* approaches: Whales and polar bear in a petri dish

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Large marine mammals fill important ecological niches as mid or top predators in marine food webs. Their high energy intake to fuel large body sizes is often accompanied with high intakes of contaminants that have bioaccumulating and biomagnifying properties. They can thus act as barometers for environmental pollution. The global burden of chemical pollution and new entities is now considered to have exceeded safe biological limits and there is thus a need for continuous and increasing efforts to monitor, mitigate, and further investigate impacts of legacy persistent organic pollutants (POPs) and chemicals of emerging concern (CECs) in wildlife. Killer whales are tope predators and by far the most contaminated marine mammal species, while considerably lower concentrations of POPs are found in the filter-feeding baleen whales. Both legacy POPs and CECs are endocrine and/or metabolic disruptors, and can negatively impact animal physiology, growth, and reproduction, as well as immune function and stem cell differentiation and hence have the potential to impair overall health of individuals and populations. To date, only a handful of studies have given mechanistic insights in contaminant response in marine mammals. Over the last couple of years, we have established alternative approaches that will allow us to address knowledge gaps using *in vitro* and *in silico* methods. The goal of this work is to characterize functional properties of key molecular targets for environmental contaminants in the killer whale (*Orcinus orca*), fin whale (*Balaenoptera physalus*), and polar bear (*Ursus maritimus*), and to establish fibroblast cell cultures from skin-blubber biopsies that can be further used in reprogramming into mesenchymal stem cell lines and in characterizing toxicological responses in the source animals. Results from ongoing studies will be presented and plans for the newly funded Marma-detox and SLICE projects will be discussed.

This work is supported by the Fram Centre Flagship-project “Cellular responses to contaminant exposure in marine mammals from the Arctic”, project no. 462/602019 to the Norwegian Polar Institute, and the SLICE project (Moving from field studies to ex vivo models for understanding and predicting toxicological responses to multiple stressors in marine mammals, NFR project no. 335489), and the Marma-detox project (Whales and polar bear in a petri dish: decoding marine mammal toxicology through in vitro and in silico approaches, NFR project no. 334739, 2023-2027) funded by the Research Council of Norway.

PFAS in drinking water in Norway; generally, very low levels but elevated concentrations were detected near known PFAS sources.

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Objectives

PFAS are contaminants that can be found in most ecosystems. Norway has had no production of PFAS, but PFOS have been used in fire-fighting foams and have been employed at a few industrial sites. In Norway, drinking water originate mostly from surface water. Norway has reported very few analytical measurements of PFAS in drinking water, and data was therefore warranted.

Methods

Drinking water from 20 different drinking sources and 11 different waterworks were sampled and sent to NIVA for analyses of 31 PFAS, including the PFAS in EU's drinking water directive and a few other PFAS groups expected to be found in water (FTS; fluorotelomer sulfonic acids and PFASA; perfluoralkane sulfonamides). Both source water (n=94) and finished drinking water (n=70) were analysed. The surface waters had a wide geographical spread east-west-south, but not northern part. Information about the technology used for treating the water was categorised (3 categories) to calculate removal efficiency (based on PFAS detected above ≥2×LOQ in source water).

Results

PFOS and brPFOS had the highest detection frequencies (95% in surface water and 89% and 94% in drinking water). Other PFAS that were frequently detected included PFBS and short PFCA (perfluorinated carboxylic acids, PFBA to PFNA) and PFHxS. 15 of the measured PFAS were not detected above the LOQ in any samples; representing mainly long PFCA and PFSA (perfluorosulfonic acids). The median number of PFAS in drinking water was 3, but up to 14 PFAS were detected in one sample.

The treatment efficiency at the waterworks were calculated based on PFAS concentrations in source water and drinking water taken the same day. The removal efficiency of PFAS for treatments with a combination of coagulation, dissolved air flotation, filtration and granulated activated carbon performed better for removing PFAS (ca. 60% removal of PFOS and brPFOS) than other categories. Details of the removal efficiency for different treatment stages at one treatment facility will be presented. Source water samples from drinking water treatment facilities not containing advanced treatment were assessed as drinking water to increase number of sample assessments vs. regulations.

EU's drinking water directive has set a limit of sum of 20 PFAS to 100 ng/L. Denmark has set a health-based drinking water limit for the sum of 4 PFAS, risk assessed by EFSA, to 2 ng/L. The newest contribution was the limits from US EPA where PFOA and PFOS had interim health advisory values of 0.004 and 0.02 ng/L respectively. All drinking water samples were below EU's drinking water limit, and all were above US EPA's limits for PFOA and PFOS. Only one sample were above the Danish health-based limit. Modelled drinking water concentrations and confidence limits for the 20 drinking water sources investigated will be presented.

Conclusion

PFAS concentrations in Norwegian drinking water are generally very low compared to concentrations reported internationally. Advanced treatment removes some PFAS, others not. Elevated concentrations in drinking water were found near known point sources of PFAS.

Integrative transcriptomics and proteomics analyses for proteogenomics genome annotation validation and mechanistic toxicology studies in Atlantic salmon

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Objectives

Develop and describe an integrative transcriptomics and proteomics analysis workflow for the efficient validation and revision of complex fish genomes. In addition, show how proteogenomics expression matrices can be used to facilitate multi-level omics data integration in non-model species *in vivo and in vitro*.

Methods

Using the Atlantic salmon (*Salmo salar*) genome as an example, we constructed proteogenomic databases from publicly available transcriptomic data of 12 salmon tissues, and in-house generated RNA-Seq data of 44 salmon liver and 60 primary salmon hepatocyte samples. Based on proteogenomics data, augmented tissue-specific protein expression matrices for liver, brain, eye, gill, gut, pyloric caecum, head kidney, heart, muscle, ovary, skin, and spleen were generated. In addition, for *in vitro – in vivo* extrapolations (IVIVE) and for the further development of new approach methodologies in salmon, proteogenomics expression matrices for 3D salmon primary hepatocyte samples were created.

Results

Our proteogenomics analysis pipeline identified nearly 80,000 peptides providing direct evidence of translation for over 40,000 RefSeq structures. The identifications included 183 co-located peptide groups ('proteogenomic events') that supported a single transcript each, and in each case, either corrected a previous annotation, supported an Ensembl annotation not in Refseq, or identified a novel (previously unannotaed) gene. Proteogenomics data derived expression matrices further revealed distinct profiles for each tissue type analyzed. Focusing on proteins involved in the defense against xenobiotics, distinct expression patterns were detected across the different salmon tissues investigated, and a large degree of homology in protein expression of chemical defense proteins was observed between *in vivo* and *in vitro* liver systems.

Conclusion

Our data corroborates the role of proteogenomic analyses in extending our understanding of complex fish genomes. We highlight the potential of proteogenomics for facilitating mechanistic toxicology studies in non-model species and show that commonly used 3D primary hepatocytes are a suitable *in vitro* model system for studying effects of xenobiotics in Atlantic salmon. Our study also provides recommendations for the incorporation of proteogenomics in future multi-level omics studies of non-model species *in vitro* and *in vivo*.

Toxicology

Interaction between selenium and methylmercury; effects on tissue levels, transcriptome and proteome in BALB/c mice.

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Objectives
Methylmercury (MeHg) is a well-known environmental contaminant, known for its toxic effects, particularly in the developing brain. The main human dietary exposure to MeHg occurs through the consumption of seafood. However, seafood also contains nutrients, such as selenium, which has previously been shown to interact with MeHg and potentially ameliorate its toxicity.
The aim of our study was to investigate the combined effects of selenium (in the form of selenomethionine; SeMet) and MeHg on accumulation of total mercury in different tissues. We also investigated the effects concomitant dietary exposure of selenium and MeHg exert on the hippocampal proteome and transcriptome of mice.

Methods
Adolescent male BALB/c mice were exposed to SeMet (2.5 mg kg^{-1}) and two different doses of MeHg (0.28 and 5 mg kg^{-1}) through their diet for 11 weeks. Organs and feces were sampled after end of the trial and analyzed for total mercury using the Direct Mercury Analyzer (DMA80). Further, hippocampi were analyzed using LC-MS/MS and RNA sequencing followed by multi-omics bioinformatics data analysis.

Results
Increased SeMet in the diet reduced the amount of mercury in several organs, including the brain, while increasing the Hg content in feces. Proteomic and RNA-seq analyses showed that both protein and RNA expression patterns were inversely regulated in mice fed SeMet together with MeHg compared to mice given only MeHg. Several pathways, proteins and RNA transcripts involved in biological processes such as immune responses and inflammation, oxidative stress, cell plasticity and markers related to Alzheimer's disease were affected inversely by SeMet and MeHg, suggesting an impact of SeMet on molecular pathways regulated by MeHg.

Conclusion
Selenomethionine can affect the accumulation and molecular effects of MeHg in a mice model.

More nutrients and less unwanted substances in organic than in conventional food

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Objectives: What is the significance of the production method for nutrients and unwanted substances in the food? The production methods within conventional and organic modes of operation, as they are practiced in Norway and internationally, are compared in terms of nutrients, additives, pesticides, pharmaceuticals and mycotoxins.

Methods: Norwegian and international literature within the field from the last decade are summarized.

Results: In Norwegian and international research, more antioxidants have been found in organic vegetables, and more polyunsaturated omega-3 fatty acids in organic milk and meat.

The regulations for organic production state that organic food must be produced with care, preferably by biological, mechanical and physical methods. Minimal use of additives and processing aids is a goal. In total, around 50 substances are approved in organic food in Norway and the EU, while around 350 substances are approved in conventional food. This means that there are generally fewer additives in organic food.

For pesticides, far fewer residues are found in organically produced vegetables - both in terms of residues above the limit values and of measurable levels below the limit values. Moreover, when detecting pesticide residues in organic samples, only one substance is consistently detected, while in conventional samples it is not unusual to find several substances.

It has been shown that there is less use of common pharmaceuticals in organic than in conventional animal husbandry. A recent English report showed four times higher antibiotic consumption in conventional animal husbandry than in organic farming. For pig production, the difference was as much as 77 times. There are no corresponding Norwegian data. A clear connection has been shown between antibiotic consumption and resistant bacteria in food-producing animals.

A recent summary of knowledge describes studies that have compared the occurrence and concentrations of the most well-known mycotoxins in grain in organic and conventional production. These toxins can be harmful to human and animal health. Most studies have demonstrated clearly lower occurrences and concentrations of the toxins in organic grain.

Conclusion: Because the occurrence and concentrations of the substances mentioned can show great variation, many studies of good quality are necessary to be able to conclude whether there are real differences between organic and conventional food. There is sufficient data to conclude that there are more beneficial nutrients and less additives, pesticide residues and mold data in organic food, as well as less antibiotic consumption and resistance in organic livestock production.

Tox

High aspect ratio nanomaterial-induced macrophage polarization is mediated by changes in miRNA levels

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Keywords: macrophage, polarization, nanomaterials, inflammation, fibrosis, epigenetic, miRNA

Abstract

Inhalation of nanomaterials may induce inflammation in the lung which if left unresolved can manifest in pulmonary fibrosis. In these processes, alveolar macrophages have an essential role and timely modulation of the macrophage phenotype is imperative in the onset and resolution of inflammatory responses. In this study, the immunomodulating effects of two classes of industrially relevant high aspect ratio nanomaterials, namely nanocellulose (NC) and multiwalled carbon nanotubes (MWCNT), and the involvement of epigenetic regulation were investigated in an air-lifted alveolar macrophage model. Our data illustrate that both nanomaterials trigger phenotypic changes in alveolar macrophages, where NC exposure leads to enhanced M1 phenotype and MWCNT promotes M2 phenotype. In accordance, MWCNT-induced M2 phenotype involved more prominent epigenetic regulatory events with changes in the expression of histone modification and DNA methylation enzymes as well as in miRNA transcript levels. MWCNT-enhanced changes in the macrophage phenotype involved prominent downregulation of the histone methyltransferases *Kmt2a* and *Smyd5* and histone deacetylases *Hdac4*, *Hdac9* and *Sirt1* indicating that both histone methylation and acetylation events may be critical in the Th2 responses to MWCNT. Furthermore, MWCNT as well as NC exposure led to altered miRNA levels, where miR-155-5p, miR-16-1-3p, miR25-3p, and miR-27a-5p were significantly regulated by both materials. PANTHER pathway analysis of the identified miRNA targets showed that both materials affected growth factor (PDGF, IGF, EGF and FGF), Ras/MAPKs, CCKR, GnRH-R, integrin, and endothelin signaling pathways. These pathways are important in inflammation or in the activation, polarization, migration, and regulation of phagocytic capacity of macrophages. In addition, pathways involved in interleukin signaling and TGFb signaling were highly enriched following MWCNT exposure. Altogether, these data support the importance of macrophage phenotypic changes in the onset and resolution of inflammation and identify epigenetic patterns in macrophages which may be critical in nanomaterial-induced inflammation and fibrosis.

Toxicology

Colorectal cancer and carcinogens related to processed meat – interactions and gender differences in A/J Min/+ mice

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Colorectal cancer (CRC) is the third most common cancer worldwide (Global Cancer Observatory, 2022). The incidence rate among Norwegian women is currently the highest in the world (Cancer Registry of Norway, 2022). Consumption of processed meat has been linked to an increased risk of CRC (Bouvard et al. 2015). Several hypotheses are proposed for the underlying mechanisms behind the carcinogenic effects of processed meat, such as presence of the meat related carcinogens polycyclic aromatic hydrocarbons (PAHs) and N-nitroso compounds (NOCs) (Demeyer et al. 2016; World Cancer Research Fund, 2018). The causality between processed meat and CRC is yet to be elucidated, and studies on interactions between diet, metabolism, microbiota, and the expression of host genes are warranted. Interactions between carcinogens can occur through multiple mechanisms, and our understanding of mixture effects is still limited. Additionally, there is an urgent need to increase our knowledge on the observed gender differences in colorectal cancer incidence.
Objectives: We aimed to investigate gender -and dose dependent carcinogenic potential of NOCs and benzo(a)pyrene (a PAH) in A/J Min/+ mice, a human relevant model for CRC.
Methods: A/J Min/+ mice were divided into 5 groups at four weeks of age (N=20 per group, both genders): control, NOCs Low, BaP Low, NOCs+BaP Low and NOCs+BaP High. All groups were fed an experimental gel diet (nocturnal exposure) 3 days per week in 9 weeks. The Low concentrations are extrapolated from a human exposure in European countries (95 percentile) and High concentrations are based on Margin of Exposure (MOE) close to 1. At thirteen weeks of age, intestines were harvested and fixated for examinations of lesions using converted light microscopy. Plasma samples were collected for targeted serum metabolomics to identify changes in serum metabolite composition as biomarkers of carcinogenic pathways.
Results: NOCs + BaP High had a significantly higher number of lesions and a higher number and load of flat aberrant crypt foci (ACF) in the colon than the other groups. A significant interaction between gender and group was found, where females in NOCs + BaP High had significantly higher number and load of lesions than the other groups. Compared to control, the other groups did not significantly affect intestinal carcinogenesis. Metabolomics results will be presented at the meeting.
Conclusion: a mixture of NOCs and BaP in a high concentration initiate and promote colorectal cancer in A/J Min/+ mice. Females are more sensitive than male mice. Combined NOCs and BaP exposure show that the carcinogens may have antagonistic effects on intestinal and colonic carcinogenesis in a gender -and dose dependent manner.

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Category: Toxicology

Characterisation and biological effects of three types of TiO_2 nanoparticles using air-liquid interface exposure

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Objectives: Titanium dioxide nanoparticles (TiO_2 NPs) are one of the most widely used nanomaterials and exposure to these nanoparticles via the respiratory route is still of major concern, especially in an occupational setting. Air-liquid interface (ALI) exposure has emerged as a valuable tool in assessing the *in vitro* toxicity of nanomaterials as it may provide more reliable data on dosimetry and prevents interactions of nanoparticles with cell culture components. The present study aimed to evaluate the biological effects of three types of TiO_2 NPs (Rutile 10-30 nm, Anatase 10-30 nm and P25 Aeroxide (anatase 80/rutile 20; 21 nm) using a complex 3D cell culture model representative of the alveolar barrier cultured at ALI.

Methods: The TiO_2 NPs were characterized using scanning electron microscopy (SEM) and dynamic light scattering (DLS). Biological contamination of the TiO_2 NPs was ruled out by using a HEKBlue reporter cell assay and Limulus amebocyte lysate (LAL) assay. Prior to nebulization and cellular exposure, the TiO_2 NPs were suspended in artificial pulmonary surfactant (Curosurf®) to better mimic pulmonary exposure scenarios. The alveolar model was exposed to relevant occupational doses, 1 and 5 $\mu g/cm^2$, of TiO_2 NPs at ALI using the Vitrocell^{TM} Cloud system (Germany). Cellular uptake of TiO_2 NPs was assessed using transmission electron microscopy (TEM). Following exposure, cell viability (using AlamarBlue) and gene expression (using Droplet Digital PCR) of genes relevant to oxidative stress responses, DNA damage and inflammation were assessed after 4h, 24h, 72h, 7 days and 14 days. Cytokine/chemokine release (ELISA) was assessed up to 72h.

Results: Using SEM, the primary particle size of all three TiO_2 NP powders was confirmed to be around 20 nm. Coating the TiO_2 NPs with Curosurf® increased the hydrodynamic diameter – measured with DLS – to 650±52 nm (Anatase), 252±1.7 nm (Rutile) and 113±2.9 nm (P25 Aeroxide), as the NPs form agglomerates in the pulmonary surfactant solution containing mostly phospholipids and hydrophobic proteins. After nebulization in the Vitrocell^{TM} Cloud, the particle diameter decreased to 65±12 nm (Anatase), 82±24 nm (Rutile) and 30±8 nm (P25 Aeroxide). Preliminary results from the Cloud exposure show that none of the TiO_2 NPs significantly affected cell viability, even after 14 days post-exposure. Gene expression analysis revealed a similar pattern for Rutile and P25 TiO_2 NPs. These particles up-regulated IFNγ at all time points and induced a strong dose-dependent up-regulation of GADD45A and CCL-2 at 14d post-exposure.

Conclusion: Altered gene expression by the TiO_2 NPs needs to be further studied to reveal the mechanisms involved. The *in vitro* alveolar barrier model at ALI combined with the coating of the TiO_2 NPs with pulmonary surfactant represents a valuable tool to assess the biological effects of these nanoparticles.

Advanced biological models in vitro for hazard assessment of nanomaterials on human health

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Advanced in vitro models are needed to pass from hazard assessment based mainly on animal studies to the application of new alternative methods (NAMs). The aim of this study was to use a 3D advanced lung model (Camassa et al., 2022) and an astrocytes-neurons in vitro model for hazard assessment of nanomaterials (NMs).

The 3D lung model of human epithelial A549 cells (1.1 x 10^5 cells/cm^2), human endothelial cells Ea.Hy926 (1.1 x 10^5 cells/cm^2) and differentiated monocytes dTHP-1 (2.2 x 10^5 cells/cm^2), were cultivated at the air–liquid interface (ALI) and exposed to 20 µg/cm^2 NM-300k (Ag-NMs) and 1 and 8 µg/cm^2 two nano-CeO_2 (3.5 and 50 nm) in an aerosol exposure system (Vitrocell® Cloud-Chamber). NM-300K was purchased respectively from Frahunofer ® and CeO_2 from Applied Nanoparticles ®.

To study systemic inflammation, conditioned cell culture medium from the previous exposures were used to expose human cerebral cell lines: astrocytes cell line 1321N1 (5 x 10^5 cells/cm^2) and neuronal cell line SH-SY5Y (5 x 10^5 cells/cm^2) cultured separately and in an astrocyte-neuronal co-culture model, as model for developmental neurotoxicity.

Advanced Respiratory Models for Hazard Assessment of Nanomaterials-Performance of Mono-, Co- and Tricultures. **Camassa LMA,** Elje E, Mariussen E, Longhin EM, Dusinska M, Zienolddiny-Narui S, Rundén-Pran E. Nanomaterials (Basel). 2022 Jul 29;12(15):2609. doi:10.3390/nano12152609.

Toxicology

Hazards associated with HDPE microplastics mixed with *Candida albicans* in granulocyte-like cells HL-60

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Objectives

Micro- and nano-plastics (MNPs) can act as vectors for toxicants like PAHs, trace metals and pathogenic microorganisms (Fabra et al., 2021; Hildebrandt et al., 2021; Trevisan et al., 2020). So far, the potential health hazards associated with mixture of high-density polyethylene microplastic particles (HDPE) and *Candida albicans* particles (CAP) remain unexplored. This study reports effects of such mixture on the viability, the pro-inflammatory responses in granulocyte-like cells differentiated from HL-60 (dHL-60) and the activation of TOLL-like receptor (TLR) 2 and 4.

Methods

To investigate health effects of HDPE (d_{50}=5 µm) mixed with CAP, we exposed dHL-60 cells to HDPE + CAP at doses varying between 0 and 200 µg/mL for HDPE and 10^3 and 10^6 CAP/mL. The effects on cell viability were assessed by AlamarBlue™ after 24- and 48-hours exposure and the proinflammatory responses measured as the levels of IL-1β, IL-6, IL-8 and TNF-α by ELISA after 6- and 24-hours exposure. Further the activation of TLR 2 and 4 was assessed using HEK 293 reporter cells.

Results

Preliminary results show no activation of TLR 2 and 4 by HDPE, but TLR 2 was activated by CAP and HDPE+CAP treatments. The viability decreases slightly with HDPE treatment. So far, only IL-8 present a signal in CAP and HDPE+CAP treated cells, compared to the negative control after 24 hours of treatment. Additionally, HDPE+CAP treated cells had higher levels of IL-8, compared to CAP treated cells.

Conclusion

Exposure of dHL60 with HDPE induced pro-inflammatory response with release of IL8 after 24h. This response increased when cells were exposed to the mixture HDPE +CAP. Furthermore, HDPE treatment slightly affects the cell viability.

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Toxicology

Hazard effects associated with HDPE microplastics combined with *Pseudomonas lurida* in human lung alveolar A549 cells

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Objective
Plastic particles have become ubiquitous and persistent in the environment. Micro- and nano-plastics (MNP) can serve as carriers for microbial pathogens (Katsumiti et al., 2021; Mammo et al., 2020), and our knowledge on health hazards associated with contaminated plastic particles remains limited. Here, we report the toxic effects of high-density polyethylene microparticles (HDPE-MPs) mixed with heat-inactivated *Pseudomonas Lurida (PL)* in human alveolar type II cells (A549).

Methods
A549 cells were exposed to HDPE-MPs (d 50= 5µm) mixed with heat-inactivated *P. lurida* at doses between 0 and 200µg/mL for the plastic particles and 0 and 2x10^6 bacterial particles for PL. Adverse outcomes, such as cytotoxicity, measured by Alamar Blue after 24h and 48h, and the release of pro-inflammatory markers, such as IL1b, IL6, IL8, and TNFα, were assessed by ELISA after 6h and 24h exposure. Furthermore, immune responses through activation of toll-like receptor (TLR) 2 and 4 by the mixture HDPE-MP + PL were also investigated.

Results
Preliminary results indicate no significant cytotoxic effect of HDPE-MP and PL, nor by the two combined. Moreover, HDPE-MP alone does not activate TLR2 or TLR4 and induces no release of pro-inflammatory markers. However, the combination of HDPE-MP +PL activates TLR2 and 4 and induces the release of IL6 and IL8 after 6h and 24h exposure.

Conclusion
Our data shows that HDPE alone does not induce cytotoxic effects or pro-inflammatory responses in A549 cells, and no activation of TLR2 or TLR4. However, the combination of HDPE-MP + PL induce pro-inflammatory and innate immune activation responses.

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Evaluation of toxicity of the microalga *Chrysochromulina leadbeateri* using the Atlantic salmon gill epithelial cell line ASG-10.

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Background:
The recent *Chrysochromulina leadbeateri* toxic bloom in Northern Norway during spring 2019[1] was disastrous for the aquaculture industry resulting in total lost production of about 40 000 tons of salmon, equating to financial losses estimated at around NOK 2.2 billion. Despite the regular and worldwide occurrence of fish-killing algal blooms, the exact mode-of-action of many potentially toxic species remains poorly understood.

Aim:
Evaluate toxic responses and investigate the mode-of-action of *C. leadbeateri* in the Atlantic Salmon gill epithelial cell line, ASG-10.

Methods:
The ASG-10 cells[2] were exposed to methanol extracts of the 2019 toxic *C. leadbeateri* strain. Toxic responses were evaluated by different viability/cytotoxicity assays (celltox green, alamar blue, caspase 3/7), ROS measurements (H2DFDA-CM probe) and morphological changes judged by light microscopy. Osmolarity of the algal media (IMR ½) were adjusted to 492 mOsmol, which seemed to be tolerable for both ASG-10 cells and the *C. leadbeateri*. The ASG-10 cells were then exposed to alive *C. leadbeateri* in IMR ½ media, and viability of the ASG-10 cells was evaluated by alamar blue and light microscopy.

Results:
The algal extracts were toxic to ASG-10 cells with an IC50 of 3.49 x 10^6 algae cells mL^{-1} after 24 h of exposure. Lower concentrations of the extract caused apoptotic cell death, while exposure to higher concentrations resulted primarily in necrotic cell death. Time-lapse microscopy revealed that cytotoxicity occurred within the first 4 h of exposure by using an extract from 4.29 × 10^6 algae cells mL^{-1}. No induction of ROS was observed. Exposure with living algae (30 000-100 000 mL^{-1}, 24–28 h) decreased viability of the ASG-10 cells. The algae did not cause any morphological changes in the ASG-10 cells.

Conclusion:
Extracts, as well as living *C. leadbeateri* reduce the viability of the ASG-10 cell line. The ASG-10 cell line seems to be a good model to study the mode-of-action of potential toxic compounds in *C. leadbeateri* algae further.

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Environmental toxicology

Cellular responses to environmental contaminants in fin whale (*Balaenoptera physalus*) primary fibroblast cell culture

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Objectives

Persistent organic pollutants (POPs) are toxic substances that can cause health hazards such as effects on reproduction, development, and the immune system. These contaminants have been a major concern since they bioaccumulate in aquatic food chains and are found at high concentrations in marine mammals. Therefore, it is of great importance to understand the molecular and cellular mechanisms of how marine mammals sense and respond to the environmental toxicants. The aim of this project is to characterize toxicological mechanisms of responses to POPs using fin whale (*Balaenoptera physalus*) primary fibroblast cell culture.

Methods

To investigate the toxicological defense system in marine mammals, we have used primary fibroblast cultures obtained from skin biopsies of fin whale. Fin whale fibroblasts were exposed to environmentally relevant concentrations of selected POPs. Following exposure to some toxicants, cell viability was measured by the lactate dehydrogenase (LDH) assay, and expression changes of some potential xenobiotic stress biomarkers were analyzed by qPCR and Western blot.

Results

- Exposure to Benzo[a]pyrene (BaP) affected neither the mRNA levels of AHRR and HSP70 nor the protein level of CYP1A1 and HSP70 in fin whale fibroblasts. However, the CYP3A protein level was upregulated upon BaP exposure.
- Our preliminary results show that exposure to mono-(2-ethyhexyl) phthalate (MEHP) increased the mRNA level of PPARG.

Conclusion

BaP is a well-established ligand of the aryl hydrocarbon receptor (AHR) that induces the expression of the target genes, such as CYP1A1 and AHRR. However, mRNA and protein levels of these genes were not altered in fin whale fibroblasts upon BaP exposure, suggesting lack of activation of the AhR pathway in these cells. On the other hand, CYP3A was upregulated by BaP exposure. CYP3A is involved in detoxification of BaP to BaP-3-ol and is normally regulated by prognane X receptor (PXR). However, it was recently proposed that the PXR gene is pseudogenized in cetaceans (Hecker et al., 2016). Therefore, the molecular mechanism of CYP3A upregulation upon BaP exposure is yet to be elucidated. In addition, the effect of other POPs, such as polychlorinated biphenyls (PCBs), will be investigated in the future work.

Acknowledgements

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Environmental toxicology

Estrogen and xenoestrogen target genes and pathways in the reproductive axis of female Atlantic cod (*Gadus morhua*).

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Objective
Estrogens regulate a range of physiological and reproductive processes in the brain-pituitary-gonadal-liver axis in fish. Although the brain and pituitary are among the main sites of action of estrogens, little work has been done on characterizing estrogen and potential endocrine disruptor effects in these organs in fish. In this work, we investigated estrogen-related target genes after exposure to xenoestrogens, including a the contraceptive pharmaceutical ethynylestradiol (EE2), the plasticizer bisphenol A (BPA) and the pesticide methoxychlor (MXC).
Methods
Juvenile female Atlantic cod were injected (*i.p*) with EE2 (10, 50 and 250 nmole/kg bw), BPA (8, 40 and 200 μmole/kg bw) and MXC (8, 40 and 200 μmole/kg bw). Three days after injection, dissection and tissue sampling was performed followed by RNA extraction. In the brain, targeted gene expression analysis was investigated using qPCR. In the pituitary and liver, comprehensive transcriptome analysis was performed using RNA-seq, followed by bioinformatics analysis. Plasma levels of vitellogenin were analyzed using ELISA.
Results
In the brain, targeted gene expression analysis showed up-regulation of the brain aromatase gene for Cytochrome P450 19a1b (Cyp19a1b) enzyme. RNA-seq analysis in the pituitary showed that the estrogenic compounds modulated the expression of many genes with reproductive roles including up-regulation of genes encoding gonadotropin releasing hormone receptor 2 (Gnrhr2), progesterone receptor (Pgr), and androgen receptor (Ar), and down regulation of genes encoding glycoprotein hormones alpha polypeptide (Cga) and the Cyp19a1b. In addition to the genes related to reproductive hormone synthesis and signaling, genes in thyroid hormone and calcium homeostasis pathways were modulated. The xenoestrogens BPA and MXC had similar effect as EE2 on the top differentially expressed genes, suggesting their potential to disrupt neuro-endocrine functions. In the liver, in addition to induction of many known vitellogenesis-related genes, a marked repressive effect of genes involved in lipid synthesis and parallel induction of lipid catabolism related genes were observed.
Conclusions
The transcriptome analysis offers new insights into mechanisms of xenoestrogen action and endocrine disruption, and further suggests candidate biomarker genes for pituitary effects. The broad estrogenic effects on diverse genes in the pituitary offer insights into how the pituitary gland might be involved in maintaining homeostasis during high plasma estrogen levels marked by vitellogenesis in the female fish.

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Environmental toxicology

Toxicity characterisation of shorter-chained and branched per- and polyfluoroalkyl substances (PFAS) by *in vivo* exposure of early stages of zebrafish (*Danio rerio*)

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Objectives

Per- and polyfluoroalkyl substances (PFAS) are a group of organic, fluorinated compounds introduced to the environment through anthropogenic activities. Their toxicity and bioaccumulative properties make PFAS a global concern, and the historically most commonly used PFAS, i.e. PFOS and PFOA, have been recognised as persistent organic pollutants and included in the Stockholm convention. So far, most toxicological studies have focused on long-chained PFAS, with far fewer focusing on the newer shorter-chained congeners. In this project, we use zebrafish to study the putative morphological effects after exposure to short-chained (PFBS, PFBA and 6:2 FTS) and branched PFAS (PFMPA**)** molecules, which were compared to longer-chained legacy PFAS (PFOS and PFOA) and the peroxisome proliferator-activated receptor (PPAR) agonist (WY14643). As several PFAS congeners have previously been demonstrated to activate the PPAR pathway in fish and mammals, changes in the expression of genes related to the PPAR signalling pathway in exposed zebrafish larvae are currently being assessed using quantitative real-time PCR (qPCR).

Methods

To investigate the toxicity of shorter chained and branched PFAS, zebrafish are exposed during their early developmental stages and morphological changes, like spine deformation and edema, are mapped with ImageJ and compared to effects induced by longer-chained legacy PFAS. RNA extraction will be conducted with 10 pooled zebrafish larvae per exposure concentration, followed by cDNA synthesis and qPCR analyses. Genes targeting the PPAR pathway will be assessed, including *pparaa*, *pparab* and *acox1*.

Results

As expected, zebrafish-larvae exposed to PFOS demonstrated different morphological changes, including spine deformation and edema. Morphological changes will later be recorded from zf-larvae exposed to shorter-chained and branched PFAS and compared to morphological changes induced by PFOA and PFOS. Preliminary assessment of RNA quality with agarose gel electrophoresis indicates low RNA degradation, and thus being suitable for qPCR.

Conclusion

The preliminary results verify that particularly PFOS induces morphological changes. While morphological effects and target genes of the short-chained and branched PFAS still need to be assessed, initial observations indicated that they are less toxic than PFOS.

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Environmental toxicology

Activation of Atlantic cod *(Gadus morhua)* retinoid X receptors by organic tin compounds

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Objectives

Organic tin compounds (OTCs), such as tributyltin (TBT) are persistant organic pollutants where Sn-atoms are bound to hydrocarbon groups. These compounds have previously been used as antifouling agents on ships and and marine installations, but is banned today due to their toxicity in numerous organisms. Retinoid X receptor (RXR) is a ligand-activated transcription factor and a member of the nuclear receptor superfamily. It is involved in several cellular processes such as development, cell differentiation, metabolism, and cell death. Furthermore, RXR is a dimerization partner for other nuclear receptors, such as Pregnane X receptor and Vitamin D receptor, and is therefore essential for their function. OTCs have been observed to act as endocrine disruptors through binding to RXR. The aim of this thesis is to study if RXR from Atlantic cod (*Gadus morhua*) can bind and be activated by different OTCs. Four subtypes of RXR have been identified in Atlantic cod (α, β1, β2, γ), which all will be studied in this project.

Methods

The ligand-binding domain of the RXR subtypes have been cloned from Atlantic cod and fused to the DNA-binding domain of the yeast GAL4 protein for studying RXR transactivation by OTCs with the GAL4-based luciferase reporter gene system in mammalian COS-7 cells. Furthermore, cytotoxicity assays and Western blot will be performed to monitor cell viability and cellular reseptor synthesis, respectively, after OTC exposure. Compounds to be tested are the RXR endogenous ligand 9-cis retinoic acid and five organic tin compounds (TBT, tripropyltin (TPT), fentin chloride (FC), fentin hydroxide (FH), trimethyltin chloride (TMTC)) as potential exogenous RXR ligands.

Results

Luciferase reporter gene assays revealed that gmRXRγ and gmRXRα were transactivated by 9-cis retinoic acid, while neither gmRXRβ1 and gmRXRβ2 were activated by this compound. gmRXRγ was potently activated by all of the OTCs, except TMTC, while gmRXRβ1 showed no response to any of these ligands (Borge, 2021). Transactivation assays with the subtypes gmRXRα gmRXRβ2 and the OTCs remain to be conducted.

Conclusion

The preliminary results indicate that all OTCs except TMTC activate gmRXRγ, and that TPT and TBT activate gmRXRα (although these are the only OTCs tested so far). There is no significant activation of gmRXRβ1 and gmRXRβ2 by OTCs, which might be due to a conserved region of 14 additional residues in helix 7 in the ligand-binding domain of both subtypes. Therefore, these gmRXR isoforms are believed to be activated through other interactions than ligand binding.

Acknowledgements

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Environmental toxicology

Dexamethasone-associated metabolic effects in Atlantic cod

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Objectives:
Dexamethasone is an often-prescribed synthetic glucocorticoid that is measured at concentrations of up to 50 ng/L in surface waters. In vertebrates, glucocorticoids regulate vital physiological activities such as metabolism, growth, hemostasis, and appetite. However, the effect of dexamethasone on these physiological processes in fish remains unclear, particularly at environmentally relevant concentrations. Here, we performed *in vivo* and *ex vivo* studies to mimic dexamethasone exposure to the ecologically and economically important species of Atlantic cod (*Gadus morhua*) and assess how this affects growth, glucose metabolism, and feeding behavior.

Methods:
To this end, we performed an *in vivo* exposure with juvenile Atlantic cod exposed to 50 ng/L, 500 ng/L, and 5000 ng/L dexamethasone and a solvent control. The groups were further divided into fed and non-fed groups. The exposure lasted for six hours every second day and was terminated after two weeks. Growth parameters were measured to evaluate the general physiological condition. Livers were dissected for gene expression analysis of gluconeogenesis-related genes. Plasma was sampled to determine glucose, triglycerides, cholesterol, and cortisol levels. In addition, a new protocol was developed to determine the metabolic profile of Atlantic cod liver tissue after exposure to dexamethasone. Precision-cut liver slices were prepared and exposed to dexamethasone before assessing the oxygen consumption rate and extracellular acidification rate using the Agilent Seahorse XFe24 system.

Results:
Comparing the physiological condition (length, weight, liver index) from before and after the exposure, no significant differences were observed between the control and exposure groups. The feeding assay showed no significant difference between the non-fed subgroups. However, in the fed group, the difference in consumed pellets, between the control and medium exposure subgroup was significant ($p<0.05$). The gene expression, biochemistry, and metabolic analysis are underway.

Conclusion:
Dexamethasone at a 10-fold concentration of concentrations measured in surface waters can reduce the feeding of juvenile Atlantic cod. This is an adverse outcome that could affect the performance and survival of exposed cod. The ongoing analysis will reveal more about the underlying mechanism.

Environmental toxicology

Does environmental transformation of engineered nanomaterials in wastewater treatment plants mitigate their availability?

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Objectives
Engineered nanomaterials (ENMs) released from an increasing number of consumer products, can end up in wastewater where they are chemically and physically transformed and removed quite efficiently by wastewater treatment plants (WWTPs). However, small amounts are still released in the environment where they might still be bioavailable. To date, most existing studies on ENMs bioavailability or accumulation were performed with pristine ENMs, high concentrations and/or the studies were conducted with artificial wastewater to simulate the transformation processes in WWTP. The aim of this study was to investigate the impact of the environmental transformation processes that occur in WWTPs at realistic ENM concentrations through the use of a stable isotope labelling technique and a pilot WWTP receiving municipal wastewater.

Methods
To investigate the fate of the ENMs within the WWTP and to produce transformed ENMs, a pilot WWTP that operated with municipal wastewater was used and spiked with isotopically enriched ENMs (^{109}Ag and ^{68}ZnO) and Au ENMs. This allows ENM detection at low concentrations in complex matrices, even against the high background levels of elements present in WWTP matrices. The effluent and sludge containing the transformed ENMs were then used to expose the benthic amphipod *Hyalella azteca* to investigate the accumulation potential of the ENMs and their respective metals.

Results
Data derived from body burden measurements showed that ^{109}Ag and ^{68}Zn from ENMs that passed through the WWTP or were incubated with WWTP matrices (e.g. spiked to control effluent) led to a strongly reduced availability in the amphipods compared to their pristine counterparts. In contrast, the accumulation of non-enriched Au ENMs in *H. azteca* was higher for ENMs that passed through the WWTP.

Conclusion
The experiments with the environmentally transformed ENMs from the pilot WWTPs showed that transformation strongly alters bioavailability and it depends on the type of metal. Environmental transformation should be taken into account for more realistic accumulation and toxicity studies.

Effect of omega-3 fatty acid intervention in vivo on energy metabolism in human skeletal muscle cells

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Keywords: skeletal muscle cells, omega-3 fatty acids, energy metabolism.

Abstract

Objective: Intake of omega-3 (n-3) fatty acids has many beneficial health effects. Positive effects of n-3 fatty acids have been shown on the body's energy conversion, including improved lipid metabolism, prevention of obesity, and improved muscle function. The primary objective of this study was to investigate the effects of n-3 fatty acid supplementation *in vivo* (1 g/d Rimfrost Krill oil) given for 7 weeks on energy metabolism and substrate turnover in muscle cells *in vitro*, isolated from biopsies obtained before and after the intervention. *Methods:* Myoblasts (proliferating satellite cells) were isolated from muscle biopsies and grown in 3-4 passages. After the proliferation phase, myoblasts were differentiated into multinuclear myofibrils (myotubes) before a high throughput assay for energy substrate uptake, turnover, and oxidation in cells was performed. Cells were preincubated with either no fatty acid (basal), palmitic acid (PA) (100 µM), eicosapentaenoic acid (EPA) (100 µM) for 24 h or high glucose (HG) (20 mM), GW (100 nM) for 96 h and insulin (100 nM) for 4 h. [^{14}C]oleic acid was used to measure fatty acid metabolism (uptake and oxidation); glucose metabolism (uptake and oxidation) was measured using [^{14}C]glucose, and protein metabolism was measured using [^{14}C]leucine. *Results:* The results showed that omega-3 fatty acid intervention *in vivo* increased glucose oxidation in cultured myotubes. Furthermore, the omega-3 intervention also increased OA oxidation in presence of high glucose and GW, glucose and leucine oxidation in response of EPA and glucose and leucine uptake in presence of PA. *Conclusion:* In conclusion, our results showed that 7 weeks of omega-3 fatty acid supplementation in healthy subjects affected energy metabolism and protein turnover in cultured myotubes.

Clinical pharmacology

Impact of type 2 diabetes on in vivo activities and protein expressions of cytochrome P450 in patients with obesity

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***Objectives*:** Previous studies have not accounted for the close link between type 2 diabetes mellitus (T2DM) and obesity when investigating the impact of T2DM on cytochrome P450 (CYP) activities. The aim was to investigate the effect of T2DM on *in vivo* activities and protein expressions of CYP2C19, CYP3A, CYP1A2, and CYP2C9 in patients with obesity.

***Methods*:** A total of 99 patients from the COCKTAIL-study (NCT02386917) were included in this cross-sectional analysis; 29 with T2DM and obesity (*T2DM-obesity*), 53 with obesity without T2DM (*obesity*), and 17 controls without T2DM and obesity (*controls*). CYP activities were assessed after the administration of a cocktail of probe drugs including omeprazole (CYP2C19), midazolam (CYP3A), caffeine (CYP1A2), and losartan (CYP2C9). Jejunal and liver biopsies were also obtained to determine protein concentrations of the respective CYPs.

Results: CYP2C19 activity and jejunal CYP2C19 concentration were 63% (-0.39 [95% CI: -0.82, -0.09]) and 40% (-0.09 fmol/µg protein [95% CI: -0.18, -0.003]) lower in *T2DM-obesity* compared with the *obesity* group, respectively. By contrast, there were no differences in the *in vivo* activities and protein concentrations of CYP3A, CYP1A2, and CYP2C9. Multivariable regression analyses also indicated that T2DM was associated with interindividual variability in CYP2C19 activity, but not CYP3A, CYP1A2, and CYP2C9 activities.

***Conclusion**:* The findings indicate that T2DM has a significant downregulating impact on CYP2C19 activity, but not on CYP3A, CYP1A2, and CYP2C9 activities and protein concentrations in patients with obesity. Hence, the effect of T2DM seems to be isoform-specific.

Clinical pharmacology and Basic pharmacology

Characterization of primary human myospheres as a three-dimensional culture model of skeletal muscle cells for metabolic studies.

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Objectives

Skeletal muscle is a major contributor to whole-body energy homeostasis and to the utilization of lipids and glucose. At present, 2D cell models have been the most used cellular models to study skeletal muscle energy metabolism. However, the transferability of the results to *in vivo* might be limited. This project aimed to develop and characterize a skeletal muscle 3D cell model (myospheres) as an easy and low-cost tool to study molecular mechanisms of energy metabolism and metabolic disorders like obesity.

Methods

Human satellite cells from *m. vastus lateralis* were isolated before and after 7 weeks of omega-3 fatty acid supplementation and cultured in 2D 96 well plates or in 3D by natural cell aggregation in U-bottom 96 well plates. Myospheres characterization was analyzed by image analysis, luminescence and qPCR. Glucose and fatty acid metabolism were studied using radiolabeled substrates and normalized by protein content measured by Bradford. Morphological and statistical analyses were performed using ImageJ and GraphPad Prism.

Results

We demonstrated that human primary myoblasts form myospheres without external matrix support and carry structural and molecular characteristics of mature skeletal muscle after 10 days of differentiation. Preliminary data showed significant metabolic differences between the models when we analyzed the effect of the omega-3 intervention. The intervention increased glucose oxidation and uptake in the cells cultivated in the 3D model. However, no effect of the intervention was observed in oleic acid metabolism or the 2D cell model.

Conclusion

These analyses demonstrate model differences that can have an impact and should be taken into consideration for studying energy metabolism and metabolic disorders in skeletal muscle.

Clinical pharmacology

Population pharmacokinetic modeling of CSF to blood clearance

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Objectives
Quantitative measurements of cerebrospinal fluid (CSF) to blood clearance have previously not been established for neurological diseases. Possibly, variability in cerebrospinal fluid clearance may affect the underlying disease process and may possibly be a source of under- or over-dosage of intrathecally administered drugs. The aim of this study was to characterize the CSF to blood clearance of the intrathecally administered gadobutrol (Gd).

Methods
Patients referred to the Department of Neurosurgery, who were examined for tentative CSF disorders and in whom intrathecal contrast enhanced MRI was considered indicated for were included. Gd was administered by intrathecal injection of 0.1-0.5 of 1 mmol/mL solution, after which blood samples and were collected up to 48 hours following administration. A population pharmacokinetic model was developed to describe individual pharmacokinetics of intrathecally administered Gd, and to determine CSF to blood clearance.

Results
Population pharmacokinetic modelling based on 1,140 blood samples from 161 individuals revealed marked inter-individual variability in pharmacokinetic profiles, including differences in absorption half-life (time to 50% of tracer absorbed from CSF to blood), time to maximum concentration in blood and the maximum concentration in blood as well as the area under the plasma concentration time curve from zero to infinity. In addition, the different disease categories of cerebrospinal fluid diseases demonstrated different profiles.

Conclusion
The present observations of considerable variation in CSF to blood clearance between individuals in general and across neurological diseases may suggest that defining CSF to blood clearance can become a useful diagnostic tool. We also suggest that it may become useful for assessing clearance capacity of endogenous brain metabolites from cerebrospinal fluid, as well as measuring individual CSF to blood clearance of intrathecal drugs.

References
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Basic pharmacology

Title: Association of early plasma cyclophilin levels with cardiovascular death in kidney transplant patients receiving calcineurin inhibitors.

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Background and Objectives
The use of immunosuppressant drugs as calcineurin inhibitors (CNI) is inevitable to prevent graft rejection after organ transplantation. However, this life-long treatment is believed to contribute to the high cardiovascular complications in these patients. Cyclophilins (CyPs) are intracellular proteins that are released upon several stress stimuli and can mediate cardiovascular diseases. CyPs were also found to be secreted in response to *in vitro* stimulation of several cell types with cyclosporine (CsA), a CNI. Therefore, we aimed to explore the plasma level of cyclophilin A (CyPA) and cyclophilin B (CyPB) in a group of kidney transplant patients treated with CNI, and evaluate their association with the incidence of cardiovascular death (CVD).

Methods
We first examined the Rikshospitalet (Oslo) registry of 1044 kidney transplant patients from 2007 to 2012 for cardiovascular death. Next, we measured the level of cyclophilin A and B in the plasma collected 10 weeks after the initiation of CNI treatment (including cyclosporine and tacrolimus) from 94 kidney transplant patients (2009) using ELISA kit. Thereafter, the relationship between plasma level of CypA and CypB and CVD complications and death were analyzed. To examine if CyPs were released as a result of cell death, the correlation between plasma CyPs and LDH was also analyzed.

Results
We found that approx. 32 % of deaths with a reported cause within 10 years after transplantation and CNI initiation were due to CVD, compared to approx. 16 % in the general Norwegian population. This result is similar to previously reported ratio in the literature (2-3 times). The mean value of both CypA and CypB level were higher in the plasma of the analyzed samples compared to the healthy donor level reported in literature. Interestingly, the mean CypA level was significantly higher in the group of patients treated with CsA compared to patient treated with tacrolimus (FK506) and the mean CypB level similar in both groups. In the group treated with CsA, the mean value of CypA level was significantly higher in patients with CVD compared to patients without CVD. When comparing the group of patients with elevated mean value of CyPA, the correlation between CyPA level and cardiovascular death was stronger. Finally, higher CypA level was not correlated with LDH, suggesting that the elevated circulating or extracellular CypA is not released after cell death, but potentially through a secretory pathway modulated by CsA.

Conclusion
Our study shows that the incidence of CVD complications in kidney transplant patients from Rikshospitalet is similar to other studies in the literature, higher than in the general population. We further found that the mean plasma level of CypA and CypB was elevated in kidney transplant patients 10 weeks after the initiation of CNI treatment and elevated CypA might be associated with the long-term cardiovascular death among patients treated with CsA.

Basic Pharmacology

Natriuretic peptides protect against apoptosis and increase cGMP around cardiomyocyte mitochondria

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Objectives

Natriuretic peptides (NPs) are known for their beneficial effects in the cardiovascular system and also for their role in regulating energy metabolism. However, little is known about the direct effect of NPs in cardiac mitochondria and possible effects on cardiomyocytes apoptosis.

Methods

We stimulated cardiomyocytes with ANP and CNP and compared results with control cells. We investigated apoptosis signaling via PARP cleavage, caspase 9 activation and cytochrome c release in the cytosol. We also constructed novel FRET-based biosensors for cGMP and targeted these to the OMM.

Results

Stimulating GC-A with ANP or GC-B with CNP reduced apoptosis PARP cleavage, together with reduced caspase 9 activation and reduced cytochrome c in the cytosol. Additionally, we show that NPs can increase phosphorylation of the pro-apoptotic protein Drp1. Using FRET biosensors, we show that both CNP- or ANP-stimulation increases cGMP locally around the mitochondria.

Conclusion

NPs are protective against apoptosis, possibly via cGMP targeting the mitochondrial outer membrane microdomain where it inhibits the pro-apoptotic protein Drp1.

Klinisk farmakologi

Impact of CYP2C19 Genotype on Escitalopram Therapeutic Failure

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Research question: Escitalopram is a selective serotonin reuptake inhibitor used in the treatment of depression and is mainly metabolized through *CYP2C19*. Genetic polymorphism in this CYP -enzyme can cause patients to achieve varying effect of escitalopram. The aim of this master thesis was to investigate the impact of *CYP2C19* -genotype on therapeutic failure of escitalopram.

Method: The study was based on patient data acquired from routine analyses at Center for Psychopharmacology at Diakonhjemmet Hospital. Patients who had measured serum concentration of escitalopram between 2010-2021 were included. Information was collected from the therapeutic drug monitoring (TDM) database, which included serum concentration of escitalopram and its metabolite, daily dose, time between last dose intake and blood sampling, age, sex, and *CYP2C19* genotype. Therapeutic failure was defined as patients who switched from escitalopram to another antidepressant within one year after the last serum measurement of escitalopram. Switching of antidepressant therapy was considered to indicate therapeutic failure either due to adverse effects or lack of pharmacological effect.

Results: A total of 20 338 patients were included in the study, whereof 4853 (23.9%) had their *CYP2C19* genotype registered in the database. In total, 1172 patients (5.8%) were identified as switchers. The percentage of switchers among the genotyped patients was 11.2% compared to 4.1% among non-genotyped patients. The degree of switching was greater in *CYP2C19* poor metabolizers (PMs) (21%), and ultrarapid metabolizers (UMs) (16%) compared to normal metabolizers (NMs) (11%) ($p<0.001$). Furthermore, the odds of switching antidepressant treatment was doubled in PMs compared to NMs (OR=2.05) ($p=0.03$).

Conclusion: This study shows that patients being *CYP2C19* PMs or UMs are more likely to switch antidepressant treatment compared to NMs, indicating that *CYP2C19* genotype is associated with escitalopram therapeutic failure.

Basic pharmacology

Cytohesins in the regulation of natriuretic peptide signalling in the heart

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Objectives

The cardiac hormones natriuretic peptides (NPs) are promising candidates for heart failure treatment. Two natriuretic peptide receptors NPR-A (activated by ANP and BNP) and NPR-B (which binds CNP) are guanylyl cyclases which produce cGMP. Previously our group demonstrated that CNP causes a negative inotropic response (NIR) and a lusitropic response (LR) due to activation of protein kinase G (PKG), and potentiates cAMP signalling through inhibition of PDE3 by cGMP. This project aimed to investigate if these effects are regulated by cytohesins, a family of proteins that activate ADP-ribosylation factors.

Methods

The expression of cytohesin 1-4 was determined in rat and mouse ventricular cardiomyocytes by Western blotting and in left ventricle tissue of healthy Wistar rats and of aorta banded and sham-operated Sprague-Dawley rats by qPCR. Interaction of cytohesin 1-4 with NPR-A and NPR-B was assessed by co-immunoprecipitation in transfected HEK293 cells, and of the purified proteins of cytohesin-2 and -4 and NPR-A and –B by microscale thermophoresis. Cyclic GMP levels were measured in rat left ventricular cardiomyocytes in the presence of the cytohesin inhibitor SecinH3 using an ELISA cGMP assay. Functional responses to NPs and cytohesin inhibition were investigated as changes in contractility of the isolated left ventricular muscle strips.

Results

We showed that cytohesins are spatially and functionally associated with NPRs in the heart. We found that cytohesin 1-4 are expressed in rat and mouse ventricular cardiomyocytes and in left ventricular tissue from rat hearts, and that in heart failure levels of cytohesin-3 and -4 increased. We demonstrated that cytohesins-2 and -4 interacted with both NPR-A and NPR-B and assessed the affinity of their binding. We showed that cytohesin inhibitor SecinH3 increased levels of cGMP produced upon activation of NPR-A, but decreased NPR-B-mediated cGMP in rat ventricular cardiomyocytes. In line with this, SecinH3 reduced the CNP-induced NIR but enhanced its lusitropic effect. Finally, on its own SecinH3 increased dF/dT_{max} and relaxation time.

Conclusion

Obtained results provide new evidence of the interaction between NPs and cytohesins and support the hypothesis that cytohesins may act as regulators of the NP system in the heart.

Clinical pharmacology

Quantifying the reactivation of MPA from MPAG – Novel method development

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***Objective*:** A large part of the considerable interindividual variability in mycophenolic acid (MPA) pharmacokinetics remains unpredictable and unexplained. The gut microbiome interacts closely with the drugs during the absorption phase and may be a significant source to explain the large difference seen in the secondary peak exposure of MPA. MPA undergoes extensive enterohepatic re-circulation by bacterial β-glucuronidase enzymes, which convert the primary metabolite of MPA, mycophenolic acid glucuronide (MPAG), back to MPA in the intestine, which then is re-absorbed and increases systemic exposure of MPA and likely enhances immunosuppression and toxicity. The effect of gut microbiota on this enterohepatic re-circulation and, thus, drug dosing is expected to be significant. The aim of this project was to develop a novel method for quantifying the reactivation of MPA from MPAG and use the method to determine MPA reactivation rates in patient samples from renal transplant recipients and healthy volunteers.

***Method*:** To identify and quantify the effects of the gut microbiome on MPA pharmacokinetic, we developed our own in-house platform of *in vitro* fecal culturing, followed by UPLC-MS analysis for monitoring the microbiome-derived metabolism of MPA. In brief, a standardized, well-characterized microbiome was incubated overnight to reach the stationary phase. MPAG was added to the inoculum and for 4 hours. Samples for drug concentration measurements of MPA and MPAG were obtained every 30 minutes. MPA concentration as a function of time was fit to a linear regression, with the slope representing the reactivation rate of MPA. The method will be used to calculate reactivation rates from renal transplant recipients included in the MicrobioTac-study, and the association with *in vivo* MPA pharmacokinetics will be explored.

Results and Conclusions: It is important to point out that this work still is in the development phase. We have managed to develop a method that quantifies the depletion of MPAG and the reactivation of MPA. Stability experiments have shown an MPAG recovery of 98% (± 0.3 %) after 24h, indicating that our results are due to metabolism carried out by β-glucuronidase enzymes and not random hydrolysis of MPAG. The preliminary results for this novel method for MPA reactivation rate will be presented at the meeting.

Basic Pharmacology for Oral Presentation

Long time delivery of C-type natriuretic peptide ameliorates cardiac diastolic dysfunction in a mouse model of heart failure with preserved ejection fraction

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Heart failure (HF) is the most rapidly growing cardiovascular health burden worldwide. Nearly 50% of these patients suffers from HF with preserved ejection fraction (HFpEF). Although several advances have been made in the understanding and treatment of HF with reduced ejection fraction (HFrEF), the majority of HFpEF patients lack effective treatment options. One of the characteristics of HFpEF is reduced diastolic filling. We have previously shown that activation of the Guanylyl Cyclase-B with C-type natriuretic peptide (CNP) increases cGMP in cardiac myocytes that leads to increased removal of Ca^{2+} into the sarcoplasmic reticulum, faster relaxation and phosphorylation of titin that leads to more compliant cardiomyocytes. We therefore hypothesized that these effects of CNP increases diastolic filling that could alleviate HFpEF. In a mouse model of HFpEF, we performed long-term treatment with CNP. To induce HFpEF, mice were fed a high fat (HFD) diet to induce metabolic stress, and administered an inhibitor of nitric oxide synthase (L-NAME) in the drinking water to induce hypertensive stress. Mice receiving maintenance diet (MD) were included as control.

To explore whether CNP prevents development of HFpEF, subcutaneous insertion of osmotic pumps containing either CNP or vehicle were performed in HFpEF and MD mice. Diastolic dysfunction was evaluated by invasive hemodynamic measurements *in vivo*. CNP treatment decreased left ventricle stiffness (EDPVR) and reduced end-diastolic pressure (EDP), thus ameliorating diastolic dysfunction. Furthermore, at a cellular level, we found that CNP-treatment improved cardiomyocyte hypertrophy. These results therefore indicates that CNP could be a future candidate for treatment of HFpEF in humans.

Impact of CYP2D6 Genotype on Paroxetine Serum Concentration

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Background: Paroxetine is a selective serotonin reuptake inhibitor metabolized by CYP2D6. Only small-scale studies have reported impact of CYP2D6 genotype on paroxetine exposure, and international guidelines differ in recommendations on whether individualized dosing of paroxetine should take CYP2D6 phenotype into account. To clarify this issue, the aim of the present study was to investigate the impact of CYP2D6 phenotype on paroxetine serum concentration in a large patient population adjusting for CYP2C19 phenotype, age and sex.

Methods: Patients were retrospectively included from a therapeutic drug monitoring database if they had measured paroxetine serum concentration and analysed CYP2D6 and CYP2C19 genotypes between 2010-2021. The impact of CYP2D6 phenotype, CYP2C19 phenotype, age and sex on paroxetine concentration to dose (C/D) ratio was investigated by multiple linear regression analysis.

Results: In total, 304 patients were included in the study; 17 CYP2D6 poor metabolizers (PMs), 114 intermediate metabolizers (IMs), 168 normal metabolizers (NMs), and 5 ultrarapid metabolizers (UMs). Multiple linear regression analysis showed that CYP2D6 IMs and PMs had 1.6-fold and 2.5-fold higher paroxetine C/D-ratio compared with NMs, respectively ($P < 0.001$). Patients who were CYP2C19 IMs or PMs had, as a merged group, 1.3-fold higher paroxetine C/Dratio vs. NMs ($P = 0.04$). Age ≥65 years was associated with a 2.0-fold increased C/D-ratio ($P < 0.001$), while sex had no significant impact on paroxetine exposure.

Conclusion: The present study shows that CYP2D6 phenotype is of significant importance for paroxetine dose requirements. CYP2D6 PMs should receive half the dosage of paroxetine compared with NMs, and for patients being both CYP2D6 PM and ≥65 years, dosing may be reduced to a fourth of normal to prevent concentration-dependent side effects. Therefore, we recommend that guidelines are clear on the importance of CYP2D6 phenotype for personalized dosing of paroxetine.

Clinical Pharmacology

Effect of fecal microbiota transplantation on CYP3A activity in patients with systemic sclerosis

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Objectives
Several studies suggests that the gut microbiota composition is involved in the expression and activity of cytochrome P450 3A (CYP3A), the single most important drug-metabolizing enzyme accounting for the metabolism of up to 50% of all drugs used in clinical practice. The gut microbiota composition in patients with systemic sclerosis (SSc) seems to differ from that in healthy individuals and these patients may therefore have an altered expression and activity of CYP3A. However, following fecal microbiota transplantation, the microbiota composition in patients with SSc may change and further lead to changes in CYP3A activity. The aim of this study was twofold; to investigate CYP3A activity in patients with SSc and both short- and long-term effects of fecal microbiota transplantation on CYP3A activity.

Method
The study was a substudy of a multicenter, randomized, double-blinded, placebo-controlled trial, including patients from 18 to 85 years with SSc, experiencing moderate to severe gastrointestinal symptoms. The included participants were randomized to receive either Anaerobic Cultivated Human Intestinal Microbiome (ACHIM) or placebo. Three 8-hour pharmacokinetic investigation days were carried out at week 0, 2, and 12. Midazolam was used as a probe drug to determine CYP3A activity. On the study days, 1.5 mg midazolam was administrated orally, followed by an individual dose of intravenous midazolam at least two hours later and in conjugation with gastroduodenoscopy. Plasma concentrations of midazolam were determined using a validated ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS).

Results
The present substudy was performed between June 1, 2021, and April 1, 2022. In total, 23 patients (1 male, 22 female) with a mean age of 58 ± 12 years were included, 22 of whom completed the study. Analyses to determine the plasma concentration of midazolam are still ongoing. Population pharmacokinetic modelling to determine absolute bioavailability and clearance of midazolam will be performed, and preliminary results will be presented at the meeting.

Clinical Pharmacology

Pharmacokinetics of unbound mycophenolic acid and the glucuronide metabolite in renal transplant recipients – implications for drug dosing

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Objectives
Mycophenolate mofetil (MMF) is widely accepted as one of the standard antimetabolites of choice in solid organ transplant treatment regimens. After oral administration, MMF is rapidly hydrolyzed to the active mycophenolate acid (MPA), which is further converted to inactive glucuronide metabolite (MPAG) via UGT enzymes. There is a large intra- and interindividual variability in MPA pharmacokinetics. Despite this, individualized dosing of MMF based on therapeutic drug monitoring is still debated and yet to be implemented at many transplant centers. Determining unbound concentrations of MPA appear beneficial as total concentrations may lead to unnecessary dose adjustments in case of changes in albumin concentrations. The aim of this study was to investigate and characterize the pharmacokinetics of unbound MPA and MPAG in renal transplant recipients included in the DayNight Study.

Methods
The DayNight study was a single-center study conducted from December 2015 until May 2017 at Oslo University Hospital, Rikshospitalet. Renal transplant recipients in the early phase post transplantation, over 18 years of age, receiving twice-daily tacrolimus based immunosuppressive therapy were eligible for inclusion. A 24-hour pharmacokinetic investigation was performed, and blood samples were collected at approximately 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 hours after the morning and evening dose of MMF and both under fasting and non-fasting conditions. Unbound concentrations of MPA and MPAG were determined by ultrafiltration before subsequent UPLC-MS/MS analysis. Standard non-compartment methods will be used to calculate the pharmacokinetic variables for unbound MPA and MPAG.

Results and Conclusion
A total of 30 patients (22 men, 8 female) with a median age of 62 (22-78) years were included in the study and 16 patients underwent two 24-hour pharmacokinetic investigations. Calculations of pharmacokinetic variables for unbound MPA and MPAG are currently under investigation. Additionally, unbound MPA concentrations from critically ill patients will be investigated in order to increase our understanding of MMF dosing in patients with low albumin levels.

Basal pharmacology

Reduced lipid metabolism and increased glucose metabolism in skeletal muscle cells from patients with spinal cord injury one year compared to one month after injury

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Backgrounds and aims

Inactivity is associated with an increased metabolic risk. Skeletal muscle adapts in response to contractile activity, allowing the muscles to more efficiently utilize substrates like glucose and fatty acids for the energy production. To characterize inactivity-related disturbances on cellular and molecular basis, we used skeletal muscle cells isolated from biopsies from subjects with spinal cord injury (SCI). People with SCI have reduced life expectancy than able-bodied population, at least partly resulting from secondary health conditions as obesity, T2D and cardio-metabolic diseases.

Methods

Human satellite cells were isolated from biopsy samples from *musculus vastus lateralis* from patients with SCI one month and 12 months after injury. The myoblasts proliferated and differentiated into myotubes, before radioactive [^{14}C] oleic acid and [^{14}C] glucose were used for studying fatty acid and glucose metabolism. Scintillation proximity assay was performed to measure incorporation and decay of [^{14}C] leucine into protein. Gene and protein expression was measured by qPCR and untargeted proteomics, respectively.

Results

Glucose uptake and oxidation were significantly increased in 12 months compared to one month-myotubes, while both oleic acid uptake and oxidation were significantly reduced. Further, 12 months myotubes had higher incorporation of leucine compared to one month myotubes. mRNA expressions of PGC1, the master regulation of mitochondrial biogenesis, and PDK4, a mitochondrial «switch» between glucose and fatty acid metabolism, as well as MYH7, coding for type I skeletal muscle fibers, were reduced at 12 months, while proteomics showed reduced expression of several mitochondrial proteins.

Conclusion

Skeletal muscle cells isolated from patients with spinal cord injury showed reduced fatty acid metabolism and reduced expression of mitochondrial proteins 12 months after the injury compared to one month. This indicates an increasing loss of oxidative capacity with time after the injury.