Project information

Keywords

polar bear, in vitro, PPAR, energetics, climate change

Project title

Contaminant effects on energetics

Year

2015

Project leader

Heli Routti

Participants

Mikael Harju, Dorte Herzke (NILU), Lisa Helgason, Kurt Kristiansen, Ingebrigt Sylte (UiT), Sabrina Tartu, Jon Aars, Eva Fuglei (NP), Anders Goksøyr, Roger Lille-Langøy (UiB),Trond Størseth (SINTEF), Anuschka Polder (NMBU), Trine Fink (Univ. of Aalborg)

Flagship

Hazardous Substances

Funding Source

2015:

FRAM: 500 000

NP: 120 000

Summary of Results

- We have studied how over 600 new and legacy contaminants bind to polar bear and human PPARs (polar bear receptors sequenced as a part of the study) using *in silico* docking and scoring methods. The results indicate that several brominated and fluorinated compounds have a potential to bind to human and polar bear PPARs. We are currently finishing testing the strong binders in vitro to confirm our in silico results.

- Activation of polar bear and human PPARs by emerging and legacy chlorinated and brominated contaminants has been studied using *in vitro* cell lines, which were established as a part of the study. The results suggest that several common pollutants found in polar bears and humans have either weak agonistic or antagonistic potential towards polar bear and human PPARs.

- Synthetic mixtures reflecting the concentrations of contaminants found in polar bear adipose tissue suppressed polar bear and human PPARγ activity as well as PPARγ-mediated accumulation of lipids in mouse fat cells. On the other hand, lipid accumulation in the mouse fat cells, through PPARγ-independent pathways was not affected following exposure to the synthetic mixtures. Contaminant mixtures extracted from polar bear tissues enhanced lipid accumulation in the mouse fat cells. We are currently characterizing a wider range of pollutants in the extracts using non-target methods.

- We have isolated polar bear stem cells from their adipose tissue and established a method to study lipid accumulation and mRNA expressions in cells exposed to mixture of contaminants. We finish the testing in January 2016. We also tried to isolate stem cells from ringed seal fat, but the method did not work for that species.

- We have determined the presence of a wide range of new and legacy compounds in polar bear fat samples by using non-target and target analyses. The will finish the target of analyses of 12 new compounds in 2016.

- Effects of contaminants on energy metabolism in polar bears are investigated using metabolomics. Polar bear samples (n=112) collected as a part of NFR project BearEnergy in spring and autumn 2012 and 2013 have been analyzed and the data handling and reporting is going on.

- We use arctic fox as a model species to investigate how fasting affects tissue distribution of perfluoroalkyl substances (PFAS), which have been reported to activate PPARs. The results show that lean Arctic foxes had increased concentrations of PFAS in adipose tissue compared to fat foxes. Additionally, concentrations of several individual PFAS in liver, kidney and blood increased with decreasing body condition.

Heli Routti has been on maternity leave for 10 months in 2015. We are therefore delayed with publications. Polar bear PPARg-manuscript has been rejected several times in high ranked journals. We are currently running more analyses and resubmitting the paper to EST early 2016.

For the Management

Highlights:

1. In silico methods indicate that several brominated and fluorinated compounds have a potential to bind to human and polar bear PPARs
2. In vitro results show that several POPs and their mixtures affect human and polar bear PPARs
3. Body condition affects PFAS concentrations. Our results using arctic fox as a model species indicate that lean foxes have higher levels of PFAS compared to fat animals.

Education:

Four students have completed their MSc-thesis as a part of the project. All have been very well evaluated (B). Two postdocs are/have been involved in the project.

Published Results/Planned Publications

Publications:

Aas CB, Fuglei E, Herzke D, Yoccoz NG, Routti H. 2014. Effect of body condition on tissue distribution of perfluoroalkylated substances (PFASs) in Arctic fox (*Vulpes lagopus*). Environmental Science and Technology. 48, 11654-11661

Castelli M, Rusten M, Goksøyr A, Routti H. 2014. mRNA expression of genes regulating lipid metabolism in ringed seals (Pusa hispida) from differently polluted areas. Aquatic Toxicology. 146, 239-246

Submitted/planned publications:

Routti H, Berg MK, Harju M, Lille-Langøy R, Rusten M, Goksøyr A. Transactivation of polar bear PPARγ and adipogenesis in 3T3-L1 cells by environmental contaminants and their mixtures. Will be resubmitted in EnvSciTechnol early 2016.

Berg MK, Lille-Langøy R, Harju M, Goksøyr A, Routti H. Comparative transactivation of human and polar bear PPARα by environmental pollutants and their mixtures. Under preparation

Helgason L, Harju M, Goksøyr A, Kristiansen K, Lille-Langøy R, Sylte I, Routti H: Theoretical models in predicting the effects of emerging compounds in human and polar bear PPARγ and PPARα). Under preparation.

Routti H, Øygarden L, Lille-Langøy R, Harju M, Goksøyr A. Adiponesis in polar bear adipose tissue-derived stem cells by mixtures of environmental pollutants.

MSc-thesis:

Øygarden L. 2015. Polar bear adipose tissue-derived stem cells as an in vitro model for effects of environmental contaminants on adipogenesis. University of Bergen.

Aas C.B. 2014. Effect of body condition on tissue distribution on perfluoroalkylated substances (PFASs) in Arctic fox (*Vulpes lagopus*). University of Tromsø.

Mari K. Berg. Peroxisome proliferator-activated receptors (PPARs) in polar bear (*Ursus maritimus)* as target receptors for environmental pollutants. 2013. University of Bergen

Martina Galatea Castelli. Effects of persistent organic pollutants in the regulation of energy metabolism in *Pusa hispida*. 2012. Università Politecnica delle Marche, Italy

Conferences:

Aas CB, Fuglei E, Herzke D, Yoccoz NG, Routti H. Body condition affects concentrations of perfluoroalkylated substances (PFASs) in Arctic fox (*Vulpes lagopus*) tissues. SETAC Europe, 25th Annual Meeting, Barcelona, Spain.3-7.5.2015. Poster

Øygarden L, Lille-Langøy R, Ruzzin J, Fink T, Aars J. Goksøyr A, Routti H. Using adipose tissue-derived stem cells from polar bears as a means to study effects of environmental pollutants. Norwegian Society of Toxicology and Pharmacology winter meeting. Beitostølen, Norway. 29.1-1.2.2015. Poster.

Aas CB, Fuglei E, Herzke D, Yoccoz NG, Routti H. 2014. Effect of body condition on tissue distribution of perfluoroalkylated substances (PFASs) in Arctic fox (*Vulpes lagopus*). Norwegian Environmental Toxicology Symposium. Stavanger, Norway, 22-24.10.2014. Talk

Routti H, Berg MK, Harju M, Lille-Langøy R, Rusten M, Ueland E, Goksøyr A. Does contaminant exposure interfere with polar bears' ability to sustain climate change? Environmental Endocrine Disruptors - An Integrated Perspective from Wildlife to Human Health. Gordon Research Conferences. Lucca, Italy, 11-16.5.2014. Talk selected from abstracts

Routti H, Rusten M, Harju M, Berg MK, Lille-Langøy R, Goksøyr A. Effect of contaminant mixtures found in polar bears on fat cell differentiation *in vitro.* Norwegian Environmental Toxicology Symposium. Tromsø, Norway, 16-18.10.2012. Talk

Castelli MG, Rusten M, Goksøyr A, Routti H. Expression of genes regulating lipid metabolism in ringed seals (*Pusa hispida*) from differently polluted areas. Norwegian Environmental Toxicology Symposium. Tromsø, Norway, 16-18.10.2012. Talk

Helgason LB, Kristiansen K, Gabrielsen M, Routti H, Lille-Langøy R, Berg M, Sylte I. In silico modeling of the peroxisome proliferator-activated receptor gamma and emerging persistent organic pollutants. Norwegian Environmental Toxicology Symposium. Tromsø, Norway, 16-18.10.2012. Talk

Berg MK, Rusten M, Eidsheim S, Lille-Langøy R, Goksøyr A, Routti H. Cloning of peroxisome proliferator-activated receptors in polar bear (*Ursus maritimus*) - target receptors for environmental pollutants? Norwegian Environmental Toxicology Symposium. Tromsø, Norway, 16-18.10.2012. Poster

Routti H. Arukwe A, Gabrielsen GW, Jenssen BM, Kanerva M, Letcher R, Nyman M, Rusten M. Contaminant effects on multiple endpoints in ringed seals - conclusions from a 5 year study. IPY 2012, Montréal, Canada, 22-27.4.2012. Poster

Routti H, Rusten M, Harju M, Goksøyr A. Effect of contaminants on polar bear fat metabolism *in vitro.* IPY 2012, Montréal, Canada, 22-27.4.2012. Poster

Popular science:

Tynne fjellrever har mer miljøgifter i kroppen. 2014. www.forskning.no

Routti H. Fuglei F. 2014. Gamle og nye miljøgifter hos fjellrev/ Old and new pollutants in arctic fox. Årsmelding /Annual Report 2014. Norsk Polarinstitutt.

Routti H. 2015. Pollutants in polar bears and other arctic animals. Fram Forum 2015. 14-17

Routti H. 2015. Miljøgifter i isbjørn. Ottar. (published in desember)

Communicated Results

We have communicated the results to management through presentations (HR) to Miljødirektoratet (Miljøgiftkonferanse 2014, Oslo) and Governor of Svalbard (Kunnskapseminar 2013, Longyearbyen). We have had an article at forskning.no and npolar.no about PFAS in arctic foxes, which caught attention in the media as well (see list under). Information about pollutants in polar bears has been published in Ottar (desember 2015) and Fram Forum 2015. We have also held presentations about pollutants in the Arctic to school classes.

PFAS in arctic foxes in web/media:

Nordnytt (NRK P1) radio (18.11.2014)

http://www.nrk.no/nordnytt/ny-miljogift-truer-reven-pa-svalbard-1.12048490

http://www.npolar.no/no/nyheter/2014/2014-11-17-ny-miljogift-fjellrev.html

http://forskning.no/miljogifter-dyreverden-svalbard/2014/11/tynne-fjellrever-har-mer-miljogifter-i-kroppen

http://www.nationen.no/politikk/miljogifta-fjellrevar-pa-svalbard/

Interdisciplinary Cooperation

This project absolutely benefits of the inter-disciplinary cooperation. The different techniques (in silico, in vitro, in vivo) are completing each other in order to get a wider understanding about contaminant effects on energetic and mechanisms involved. This project includes both physical and analytical chemistry, molecular and cell biology, physiology and ecology.

Budget in accordance to results

Fram Centre funding 2015 has covered data analyses of metabolomics at SINTEF and approximately one third of reporting time (Sabrina Tartu). The funding also has covered over a half of costs for in vitro analyses on polar bear stem cells and characterization of tissue extracts (salary to Lene Øygarden, laboratory equipement, use of laboratory, analyzing of polar bear tissue extracts. Fram Centre funding covers target analyses of new pollutants in polar bear tissues. Selection of the pollutants is based on non-target analyses payed by NP.

NP has covered salary costs of Heli Routti.

Fram Centre funding has been necessary and sufficient.

Could results from the project be subject for any commercial utilization

No

Conclusions

Future perspectives:

In the present study we have had a lot of weight on investigatin pollutant effects in vitro. To increase our understanding of how contaminant exposure is linked to energy metabolism and lipid homeostasis we need now more knowledge what happens *in vivo.* Metabolomics data in free-ranging polar bears (part of the present study) in addition to mRNA expression of PPARγ and its target genes in relation to contaminant exposure (BearEnergy/NFR) deepens our understanding on the physiological effects of pollutants in polar bears. It is also of interest to widen the persepective of interactions between pollutants and climate change to a more ecological level.

New methods:

In silico: We have built up an extensive library of new chemicals for this project that can now been easily used for other in silico studies.

In vitro: The extraction method used in this study by NILU is a new combination of known methods. The methods used in *in vitro* preadipocyte studies have previously been used by other laboratories; the methods have been implemented to UiB due to this project. Two new methods of has been established at UiB, namely luciferase reporter assay to include polar bear PPARs and a method to study lipid accumulation and mRNA expression in polar bear stem cells isolated from adipose tissue. These methods are highly valuable tools to assess the metabolic effects of pollutants.