FACULTY OF VETERINARY MEDICINE
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ASSESSMENT OF CHEMICALLY INDUCED GENOTOXICITY AND CANCER RISK BY MEANS OF **DNA ADDUCTOMICS**

DNA adductomics platform

Methodology, Technology & Tools

The exposure of DNA to both endogenous and exogenous genotoxic chemicals can result in the formation of DNA adducts and is believed to be the **first step in chemically induced carcinogenesis**. DNA adductomics is a fairly recently breached branch of metabolomics which studies the formation of DNA adducts as a result of exposure of cellular DNA to a wide variety of chemicals in day to day life. To facilitate **targeted as well as untargeted DNA adduct analysis**, we optimized and validated a unique state-of-the-art Ultra High Performance Liquid Chromatography - **High Resolution Mass Spectrometry** based methodology using hybrid **Quadrupole-Orbitrap** technology (Q-Exactive™). In addition, to allow in-depth investigation of diet-related DNA adduct formation, a **DNA adduct database**, containing more than 150 known diet-related DNA adducts, was constructed (L.Y. Hemeryck et al., 2015, *Anal Chim Acta*).

Current research focus

In 2015, the International Agency for Research on Cancer and the World Health Organization issued that **red meat is 'probably carcinogenic to humans'** and processed meat is 'carcinogenic to humans'. More specifically, epidemiological research has demonstrated that red meat consumption significantly contributes to **colorectal cancer** (CRC) risk. Different hypotheses have been put forward to explain this causal relationship but the **heme hypothesis**, stating that heme iron present in red meat stimulates the formation of **genotoxic N-nitroso compounds (NOCs) and lipid peroxidation products (LPOs)**, has received the most support. Both NOCs and LPOs can exert DNA damaging effects, but the exact underling mechanisms of red meat and heme iron genotoxicity still require further elucidation. In light of this, we successfully applied the in-house DNA adductomics platform to investigate red meat induced DNA adduct formation in several *in vitro* and *in vivo* experimental setups.

Workflow

1. **DNA hydrolysis**
   0.1 M FA at 80 °C during 30 min
2. **DNA adduct extraction**
   SPE: Oasis HLB 1cc, 30 mg
3. **UHPLC-HRMS analysis**
   Q-Exactive: Quadrupole-Orbitrap
4. **Data processing & interpretation**

Targeted
DNA adduct analysis:

By means of Xcalibur™ software

❖ NOC-related DNA adducts:
- O^6-carboxymethylguanine
  O^6-methylguanine

❖ LPO-related DNA adducts:
- Pyrimidopurinone
- Methylhydroxypropanoguanine

Untargeted
DNA adductome mapping:

By means of ToxFinder™, Sieve™ & Simca™ software

❖ ToxFinder™:
- Match *m/z* of retrieved ions to in-house DNA adduct database
- Univariate statistics

❖ Sieve™ & Simca™:
- Alignment and framing of ion intensities
- Multivariate statistics (OPLS-DA)
- Match *m/z* of relevant ions to in-house DNA adduct database

Case study: rat feeding trial

Experimental setup

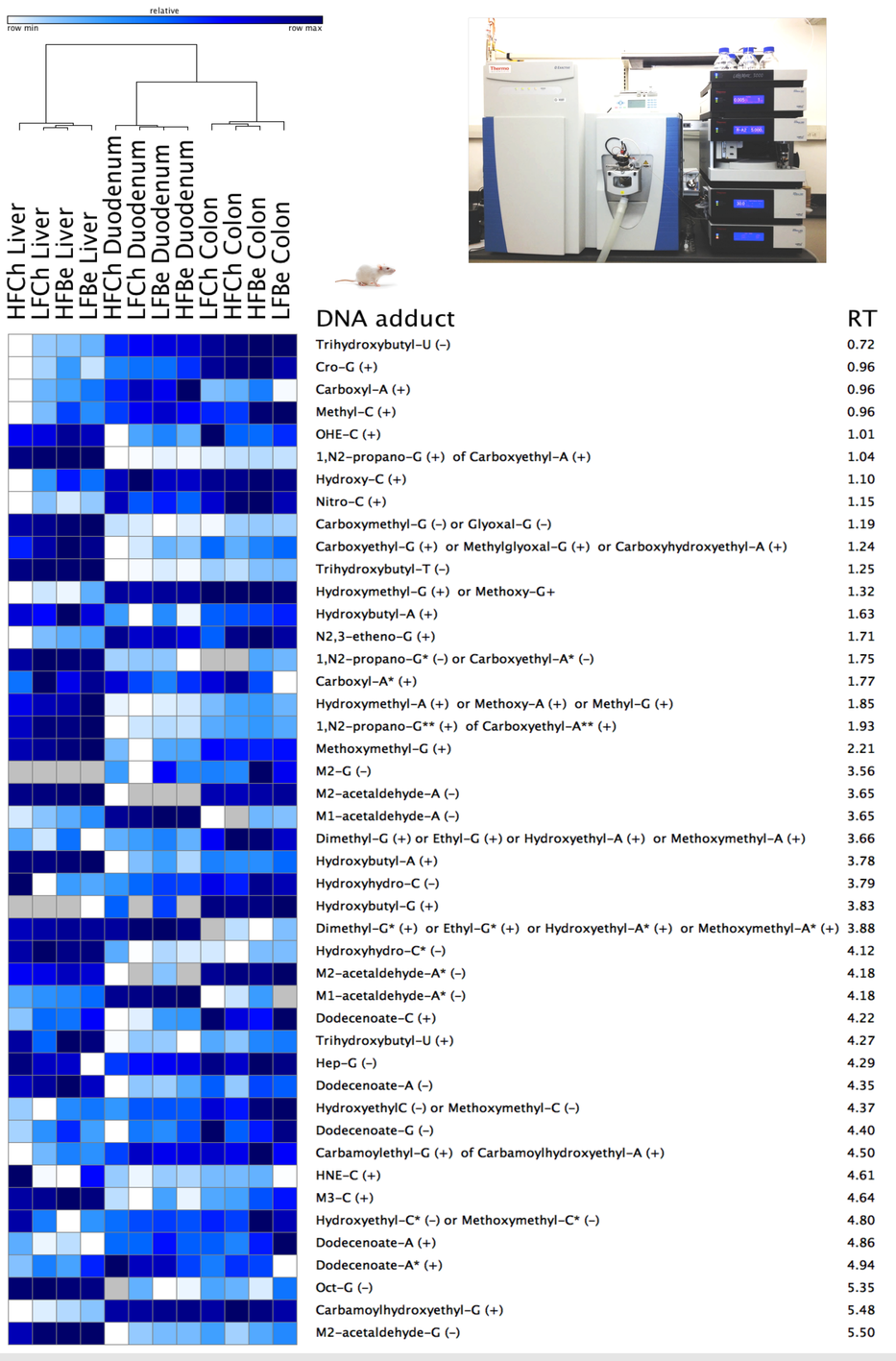
24 **Sprague-Dawley rats** were divided into 4 randomly composed groups and either fed a **low or high fat beef diet**, **or a low or high fat chicken diet** during 14 consecutive days. After tissue sampling, the DNA adductome of liver, duodenum and colon were mapped.

DNA adduct profiling

Figure on the right:
Heat map of average (n=6) DNA adduct types and levels in rat liver, duodenal and colon DNA. **Darker shades of blue represent higher average DNA adduct levels**. RT represents retention time in min., an asterix marks a different isomer of a certain DNA adduct that had already been detected at an earlier RT. A grey box represents the total absence of DNA adduct detection in those particular samples. The ionization mode in which each DNA adduct type was detected, is provided between brackets after each DNA adduct name.

Results

Extensive data processing by means of different 'omics' software packages allowed a thorough evaluation of the (significant) effects of the digestion of the different meat based diets on the DNA adductome in rat liver, duodenum and colon. We observed that the **DNA adductome differed in each tissue type**. More importantly, rats on a **different meat diet** demonstrated a **different DNA adduct profile**. **Intake of beef and/or lard induced a significant rise in the levels of 22 DNA adduct types**. These particular types of DNA adducts were formed due to alkylation and/or oxidation of DNA nucleobases. As such, their formation is of particular interest to the red and processed meat-CRC hypotheses.



Obtained output

(Markers for) red meat genotoxicity

Via the execution of **several independent red meat genotoxicity studies (*in vitro* & *in vivo*)** (Vanden Bussche et al., 2014, *Mol Nutr Food Res*; Hemeryck et al., 2015, *Toxicol Res*; Hemeryck et al. 2017, *Food Chem*; Hemeryck et al., 2017, *Food Chem Toxicol, subm.*), the in-house DNA adductomics platform has allowed us to study red meat induced genotoxicity and pinpoint **7 DNA adducts with** significant **marker potential** (listed in the table below).

DNA adduct type	DNA adduct origin
O^6-Carboxymethyl-G	Alkylation
Dimethyl-T or ethyl-T	Alkylation
Methyl-G	Alkylation
Malondialdehyde-2x-G	Lipid peroxidation
Heptenal-G	Lipid peroxidation
Carbamoylhydroxyethyl-G	Alkylation
Malondialdehyde-3x-C	Lipid peroxidation

Future perspectives

❖ **Human *in vivo* confirmation** of the obtained results (case-control CRC study + dietary intervention study).
❖ Integration of the DNA adductomics platform with Metabolomics, Lipidomics & Metagenomics
➔ **Fused omics**

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