DNA ADDUCT MARKERS ASSOCIATED WITH THE GASTROINTESTINAL DIGESTION OF RED MEAT

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Estimated number of incidence cases, both sexes, worldwide (top 10 cancer sites) in 2012
Estimated age-standardized rates (global) of incidence, both sexes, colorectal cancer, worldwide in 2012.
## COLORECTAL CANCER (CRC) RISK

<table>
<thead>
<tr>
<th>Factors that increase risk</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol consumption (heavy vs. nondrinkers)</td>
<td>1.6</td>
</tr>
<tr>
<td>Obesity</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Red meat consumption</strong></td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Processed meat consumption</strong></td>
<td>1.2</td>
</tr>
<tr>
<td>Smoking (current vs. never)</td>
<td>1.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Factors that decrease risk</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical activity</td>
<td>0.7</td>
</tr>
<tr>
<td>Dairy consumption</td>
<td>0.8</td>
</tr>
<tr>
<td>Fruit consumption</td>
<td>0.9</td>
</tr>
<tr>
<td>Vegetable consumption</td>
<td>0.9</td>
</tr>
<tr>
<td>Total dietary fiber (10 g/day)</td>
<td>0.9</td>
</tr>
</tbody>
</table>
RED VS. WHITE MEAT: HEME HYPOTHESIS

Myoglobin containing heme
- Passage through gastrointestinal tract
- Non-absorbed fraction

→ Passage through ascending, transverse and descending colon: catalyzes a number of endogenous transformations
HEME IRON TOXICITY

Heme iron in the gut

Direct toxicity
- Cytotoxic
- ?

Indirect toxicity
- Stimulation of \(N\text{-nitroso compound (NOC)}\) formation
- ?
- Stimulation of (lipid per)oxidation (LPO)
- Genotoxic
- Cytotoxic & Genotoxic

Cancer initiation, promotion and progression
STUDY GOALS

1. Install a UHPLC-HRMS based DNA adductomics methodology
   → To facilitate targeted as well as untargeted DNA adduct analysis

2. Study differences in DNA adduct formation due to red vs. white meat digestion
   a. Effect of calcium (cancer-protective attributes)
   b. Effect of myoglobin (heme iron)
   c. Effect of lower vs. higher dietary fat content (Western diet)
UHPLC-HRMS DNA ADDUCTOMICS

- Accurate mass measurements
- **High specificity** ➔ identification with high certainty
- **High sensitivity** ➔ quantification of low levels

**Optimisation:**

Targeted & untargeted DNA adduct detection

Quadrupole-Orbitrap (Q-Exacte™)

**Successful validation:**

Hemeryck et al., 2015, *Analytica Chimica Acta*
RED VS. WHITE MEAT (1): IN VITRO DIGESTION MODEL

Vanden Bussche et al. 2014
Molecular Nutrition and Food Research
RED VS. WHITE MEAT (2): **IN VIVO** RAT MODEL

- **14-day feeding trial**

  ![Image](image.png)

  OR

  - Sampling of liver, small and large bowel tissue
    - Extraction of DNA and DNA adducts
    - DNA adduct analysis
CONDUCTED EXPERIMENTS & STUDIES

1. *In vitro* digestion of chicken, pork & beef
   - 15 fecal inocula
   - Limited to targeted DNA adduct analysis

2. *In vitro* digestion of chicken & beef
   - 5 fecal inocula
   - Targeted & untargeted DNA adduct analysis
   - Additionally: assessment of effect of calcium (CaCO$_3$) addition

3. *In vitro* digestion of chicken & beef
   - 10 fecal inocula
   - Targeted & untargeted DNA adduct analysis
   - Additionally: assessment of effect of myoglobin addition

4. *In vivo* digestion of chicken & beef
   - 14-day feeding trial
   - Sprague-Dawley rats
   - Targeted & untargeted DNA adduct analysis
   - Additionally: assessment of effect of lard content
1.1 *In vitro* digestion of beef using 5 different fecal inocula

→ DNA adduct formation?

1.2 Selection of 2 fecal inocula for further investigation:

Beef vs. Chicken & CaCO$_3$ supplementation

→ DNA adduct formation?
DNA adduct formation upon the *in vitro* digestion of beef using 5 different fecal inocula: P1-P5

Pre-colonic levels subtracted from post-colonic levels = representation of in- or decrease during colonic fermentation

Interindividual variation

Some DNA adduct types rise, whilst others decrease during colonic fermentation
DNA adduct formation upon the *in vitro* digestion of different meat types using 2 different fecal inocula: P1 & P2

Comparing:
- Beef vs. chicken
- Non-supplemented beef or chicken meat vs. beef or chicken supplemented with CaCO₃

In (pre- and) post-colonic digestion samples

⇒ Meat type strongly influences DNA adduct formation
DNA adductomics to study the genotoxic effects of red meat consumption with and without added animal fat in rats

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- \textit{In vivo} digestion of beef or chicken by Sprague-Dawley rats

- differences in DNA adduct levels in liver, duodenal and colonic tissue?

+ Investigation of the interfering role of dietary fat
DNA adduct formation in liver, duodenum & colon upon digestion of:

- a low fat beef diet (‘LFBe’), or
- a low fat chicken diet (‘LFCh’), or
- a high fat beef diet (‘HFBe’), or
- a high fat chicken diet (‘HFCh’)

- Prominent difference according to tissue type
- Difference according to diet
- 22 DNA adduct types increased due to beef and/or lard digestion
## DNA ADDUCTS WITH RED MEAT MARKER POTENTIAL

<table>
<thead>
<tr>
<th>DNA adduct name</th>
<th>DNA adduct type</th>
<th>Context</th>
<th>Test</th>
<th>p-value or VIP score</th>
</tr>
</thead>
<tbody>
<tr>
<td>O⁶-Carboxymethyl-G</td>
<td>DNA alkylation</td>
<td><em>In vitro</em> (x3)</td>
<td>ANOVA &amp; t-test</td>
<td>p = 0.05, p &lt; 0.01, p = 0.05</td>
</tr>
<tr>
<td>Dimethyl-T or ethyl-T</td>
<td>DNA alkylation</td>
<td><em>In vitro</em> (x2)</td>
<td>Sieve™ pairwise comparison &amp; Simca™ analysis</td>
<td>p = 0.02, VIP = 1.95</td>
</tr>
<tr>
<td>Methyl-G</td>
<td>DNA alkylation</td>
<td><em>In vitro</em> (x2)</td>
<td>Simca™ analysis &amp; t-test</td>
<td>VIP = 1.23, p = 0.03</td>
</tr>
<tr>
<td>Malondialdehyde-2x-G</td>
<td>Lipid peroxidation &amp; attack of DNA</td>
<td><em>In vitro &amp; in vivo</em></td>
<td>Sieve™ pairwise comparison &amp; GENE-E marker selection</td>
<td>p = 0.05, p = 0.02</td>
</tr>
<tr>
<td>Heptenal-G</td>
<td>Lipid peroxidation &amp; attack of DNA</td>
<td><em>In vitro &amp; in vivo</em></td>
<td>t-test</td>
<td>p = 0.05, p = 0.03</td>
</tr>
<tr>
<td>Carbamoylhydroxyethyl-G</td>
<td>DNA alkylation</td>
<td><em>In vitro &amp; in vivo</em></td>
<td>t-test</td>
<td>p = 0.03, p = 0.04</td>
</tr>
<tr>
<td>Malondialdehyde-3x-C</td>
<td>Lipid peroxidation &amp; attack of DNA</td>
<td><em>In vitro</em> (x2)</td>
<td>Sieve™ pairwise comparison &amp; t-test</td>
<td>p &lt; 0.01, p = 0.01</td>
</tr>
</tbody>
</table>
CONCLUSIONS: RELEVANT TO RED MEAT-CRC LINK?

Red meat/heme iron digestion

- Stimulation of N-nitroso compound (NOC) formation
  - Genotoxic
    - Methyl-G
    - O6-carboxymethyl-G
    - Dimethyl-T or ethyl-T
    - Carbamoylhydroxyethyl-G

- Stimulation of (lipid per)oxidation (LPO)
  - Cytotoxic & Genotoxic
    - DNA adduct formation
    - Heptenal-G
    - Malondialdehyde-2x-G
    - Malondialdehyde-3x-G

- ? Unknown ?

Cancer initiation, promotion and progression
Polar metabolomics – chemical targets

- Amino acids
- Amines
- Other N-compounds
- Polyols
- Bile acids
- Carbohydrates
- Short chain fatty acids
- Hydroxy acids
- Multicarboxyl acids
- Monocarboxyl acids
- ...

Lipidomics – chemical targets

- Fatty acyls
- Phospholipids
- Prenols
- Sterols
- Glycerolipids
- Glycerophospholipids
- Polyketides
- Sphingolipids
Discovery of 5 discriminating metabolites with potential involvement red meat related diseases

- 3-dehydroxycarnitine
- Dityrosine
- Kynurenine
- N’-formylkynurenine
- Kynurenic acid

PARALLEL RESEARCH: METABOLOMICS RED VS. WHITE MEAT
FUTURE RESEARCH: FUSED OMICS

METADATA
- Age
- Gender
- Dietary habits
...

FUSED OMICS platform
- Data processing
- Data integration
- Pathway analysis
- Biomarker discovery

METABOLOMICS
- Lipids
- Polar metabolites

METAGENOMICS
- Composition of the gut microbiome

DNA ADDUCTOMICS
- Chemically altered DNA nucleobases
Thank you!

Lynn Vanhaecke
Prof. Dr.

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