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Mapping of the DNA adductome to study the genotoxic effects of red meat consumption

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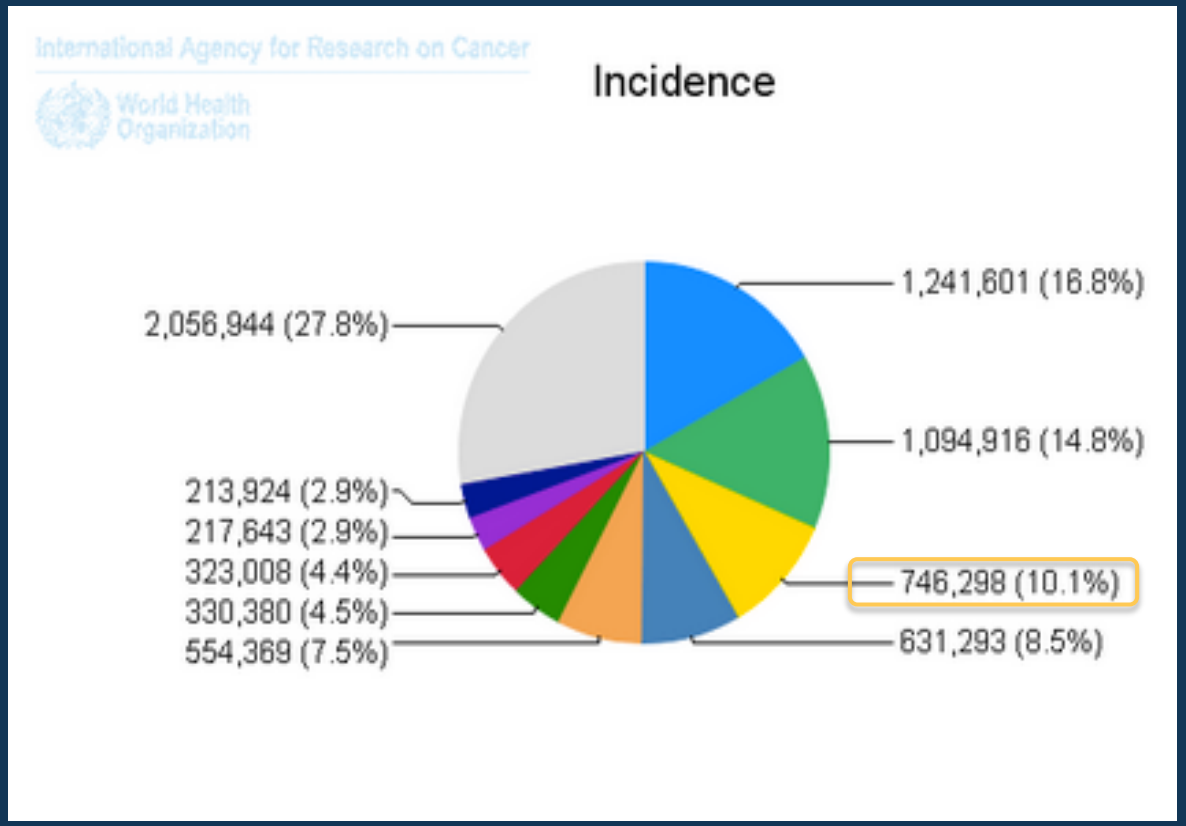
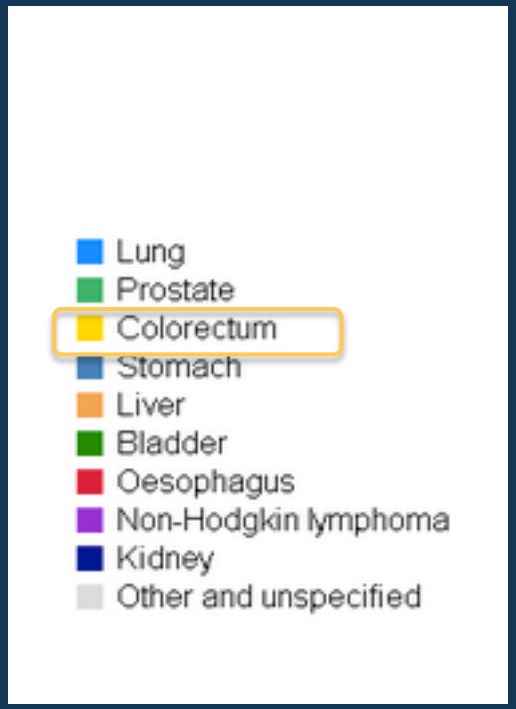
NuGO week 2015 Barcelona, Spain

Worldwide cancer incidence



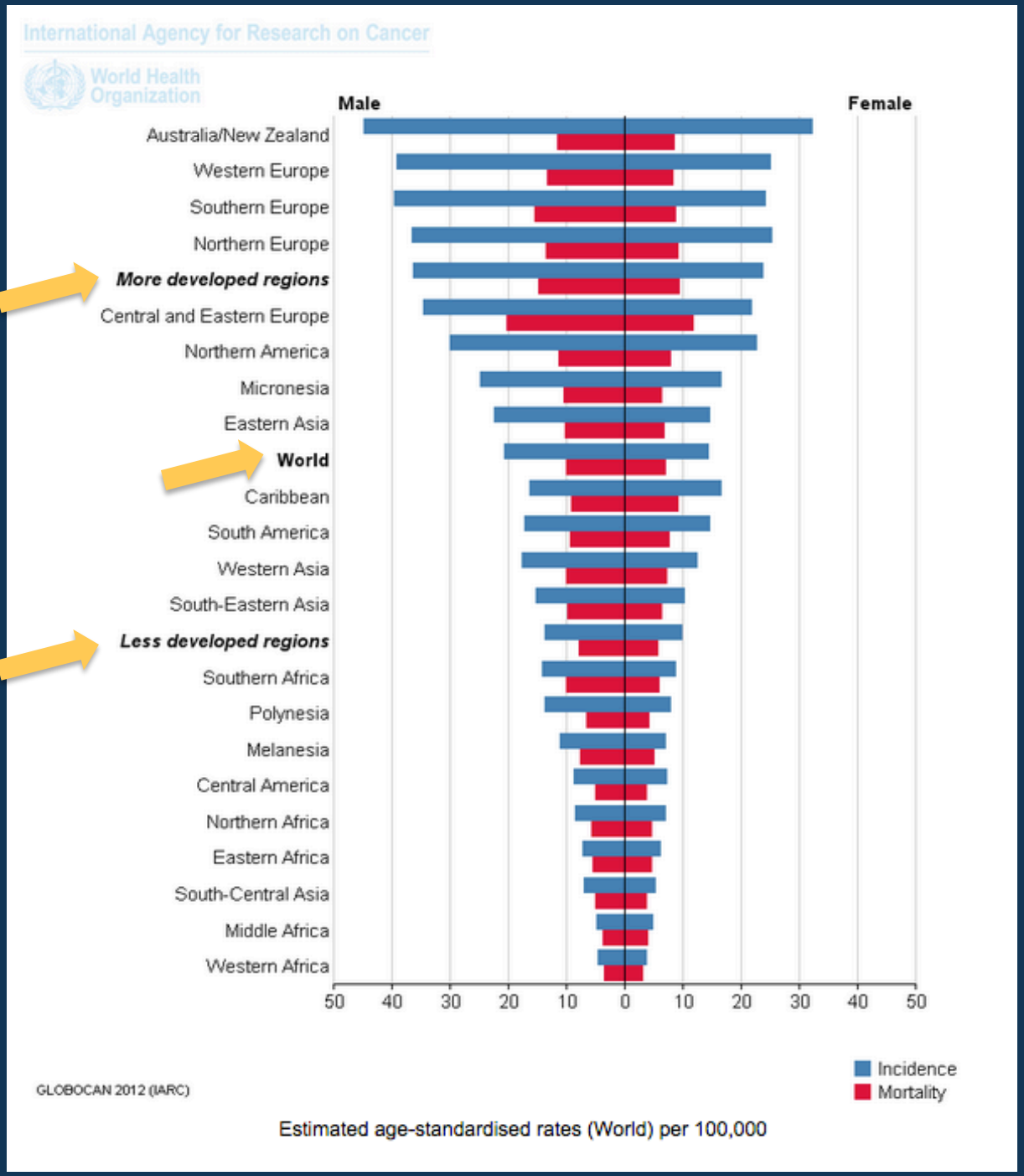
Globocan 2012 (IARC)



Colorectal cancer risk factors

- ≅ 10 % genetic
- ≅ 90 % non-genetic
  - – Tobacco
  - – Alcohol
  - – Unhealthy diet
  - – Physical inactivity
  - – …

Doll & Peto, J. Natl. Cancer Inst., 1981
Willett, Environ. Health Perspect., 1995



Globocan 2012 (IARC)

Colorectal cancer incidence rates per 100,000 men/women worldwide

Colorectal cancer risk factors

Western-type diet

80 %

Unhealthy diet

Doll & Peto, J. Natl. Cancer Inst., 1981
Willett, Environ. Health Perspect., 1995

Epidemiological data

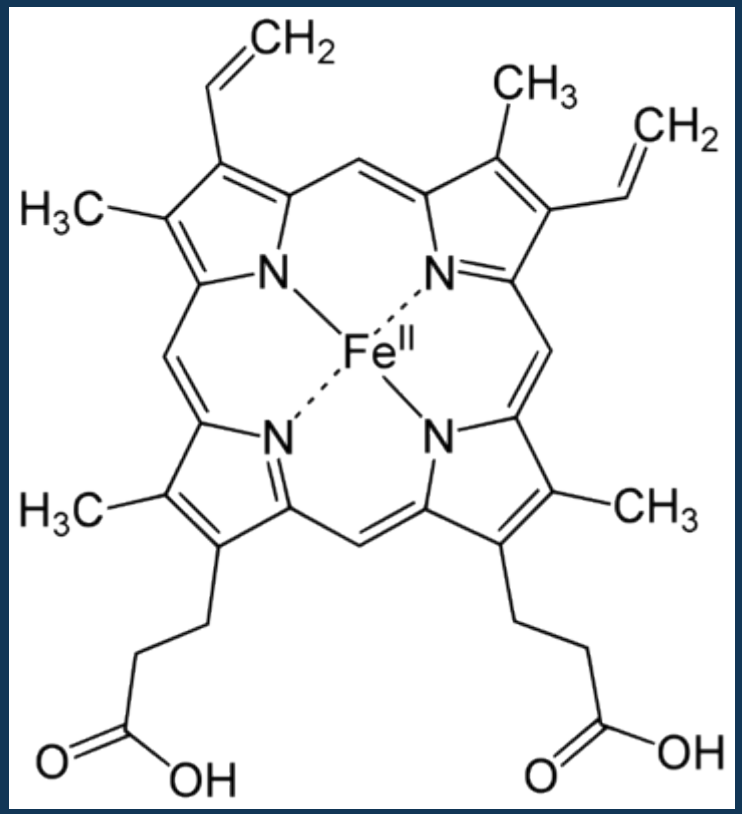
100 g / day → Increased risk of 29 %

50 g / day → Increased risk of 21 %

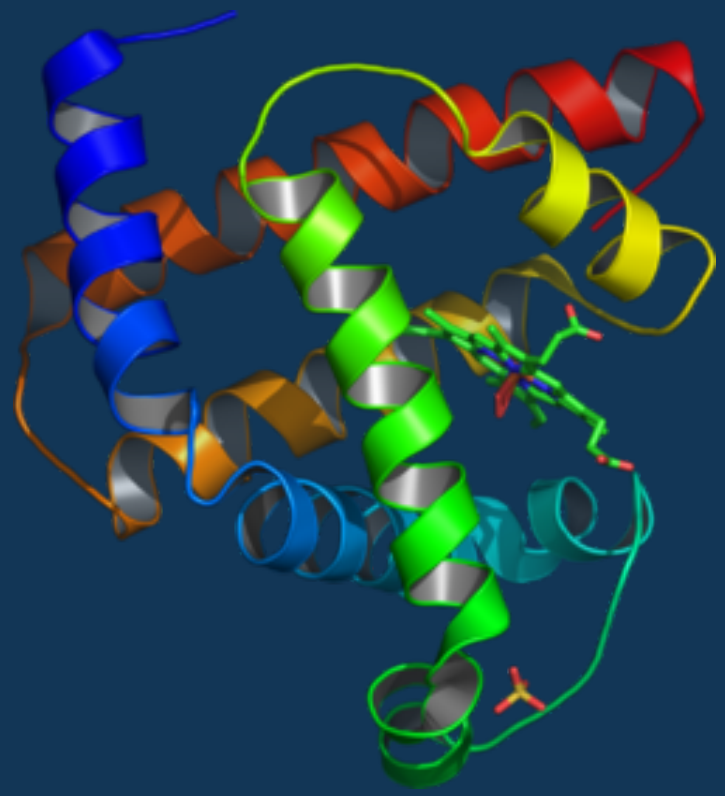
WCRF, 2007

Red meat and colorectal cancer hypothesis

Haem!



Myoglobin

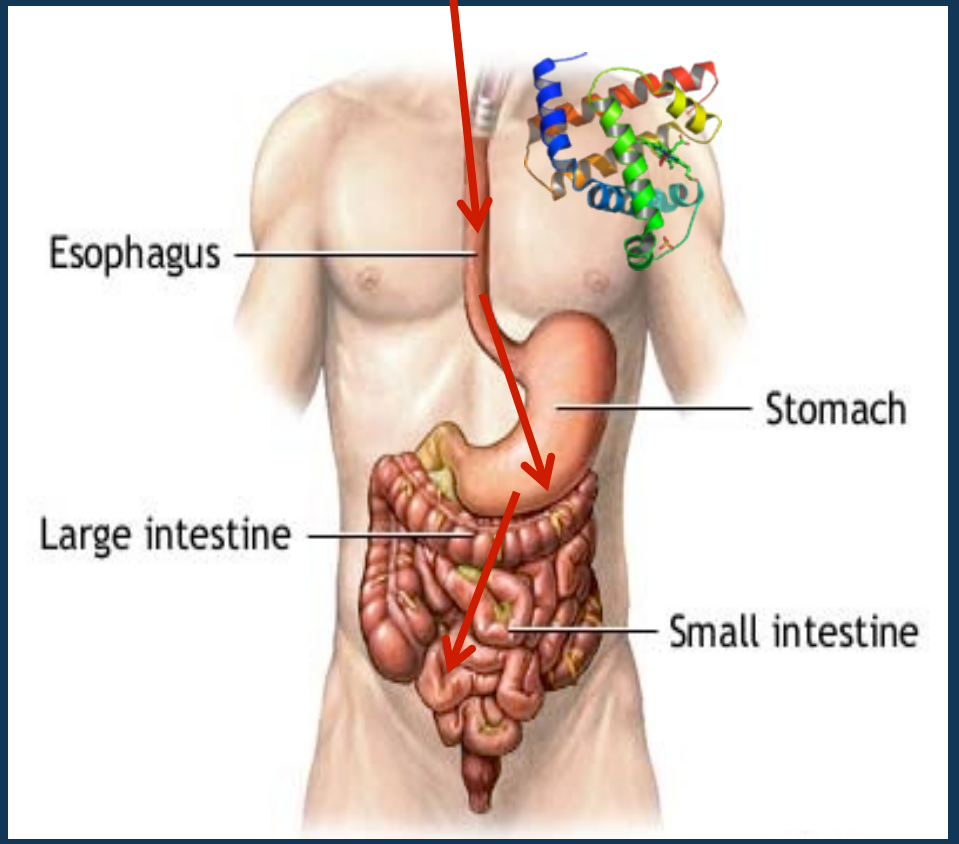


Oostindjer *et al.*, Meat Science, 2014

Red meat *vs*. colon cancer: role of GI red meat digestion?

Meat with haem pass through GIT and stimulate formation of:

1. Lipid peroxidation products (LPOs)
2. N-Nitroso compounds (NOCs)

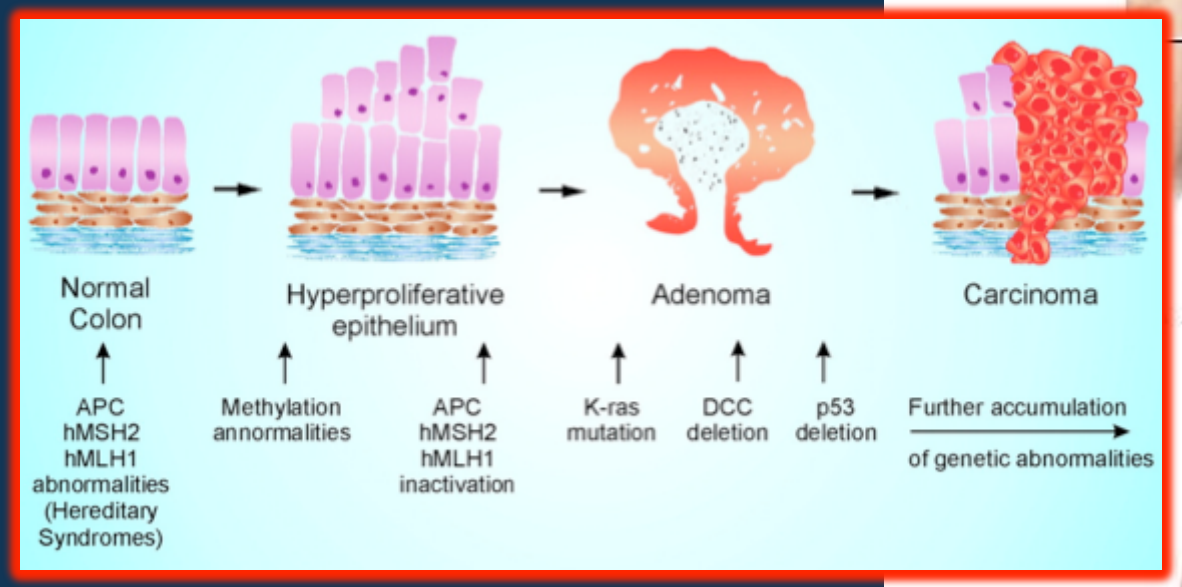


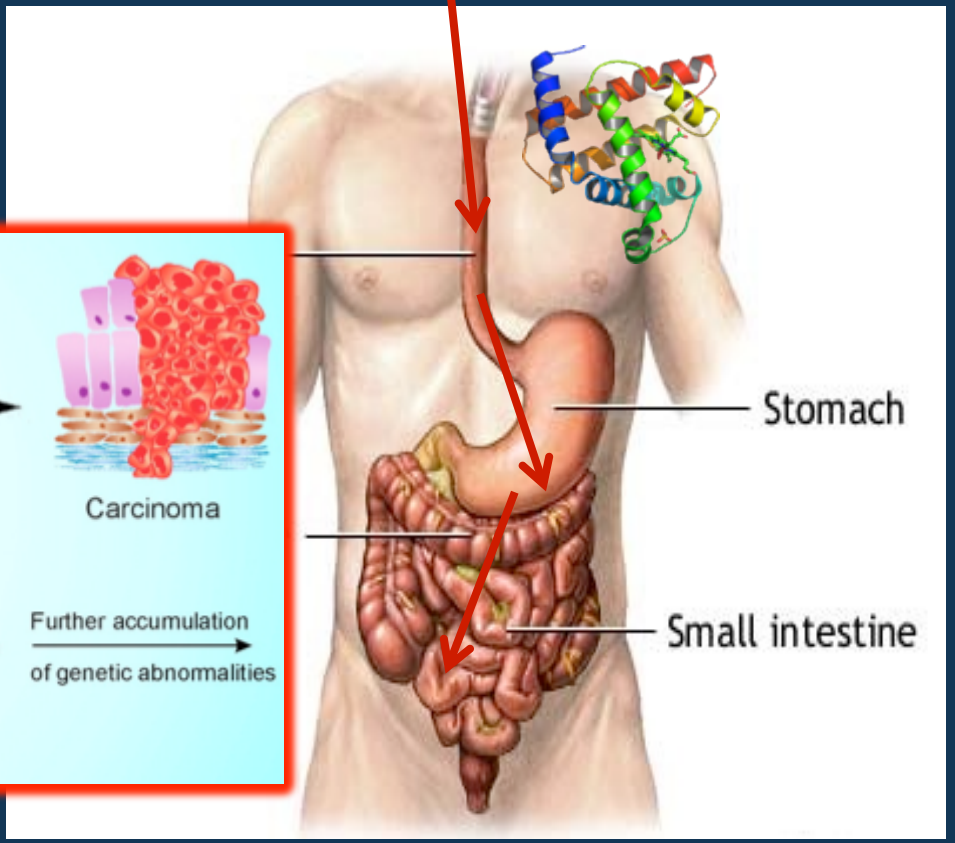
Red meat *vs.* colon cancer: role of GI red meat digestion?

NOCs and LPOs

DNA damage **DNA adducts**

Mutations
K-*RAS*, p53



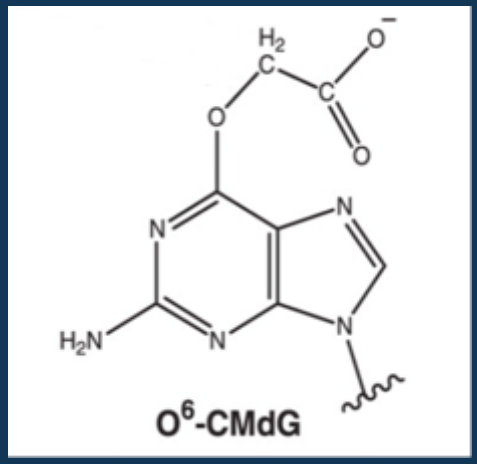


Experimental setup

1. *In vitro* digestion of red (vs. white) meat
2. Mapping of DNA adducts by means of UHPLC-HRMS in meat digests

DNA adduct?

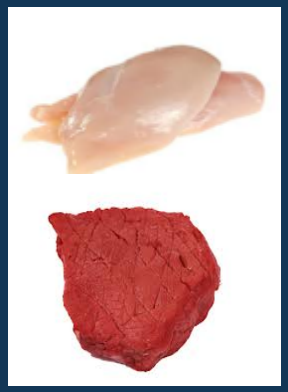
- When a chemical covalently binds to a DNA nucleobase
- First step in chemically induced carcinogenesis
- Different types:
  - By NOCs
  - By LPOs
  - And many others…



In vitro digestion of meat

STATIC *IN VITRO* DIGESTION (37°C)

Grinding and cooking



Stomach (2h)

Small intestine (4h)

Large intestine/colon (72h)

Colonic microbiota of volunteers

Gastro-intestinal juices

DNA adduct analysis: DNA adductome mapping

1. Sample preparation:
   a. Acid DNA hydrolysis (0.1 M HCl)
   b. Solid Phase Extraction
2. UHPLC-HRMS analysis
   a. Targeted (“profiling”)
   b. Untargeted (“fingerprinting”)
3. Data processing
   a. ToxID™
   b. SPSS®
   c. Sieve™
   d. Simca™

Results (1):

- What?
  - Digestion of beef
  - Colonic digestion by means of the colonic microbiota obtained from 5 different volunteers
  - Increase, decrease or shift during colonic digestion?

- How?
  - ToxID™ data processing based on *m/z* of known diet-related DNA-adducts

- ❑ N.D.
- ❑ Rise in DNA adduct levels:
  DNA adduct formation
- ❑ Decrease in DNA adduct levels:
  DNA adduct degradation or dilution
- ❑ *(*)(*)(*) isomers

Results (2):

- What?
  - Digestion of beef *vs.* chicken
  - *+ vs.* with or without added calcium
  - Microbiota of 2 volunteers:
    - P1 & P2
    - 3 biological replicates
  - Increase, decrease or shift?
- How?
  - ❖ ToxID™ data processing

- ❑ N.D.
- ❑ Beef (B) > Chicken (C) OR beef/chicken without added calcium > with added calcium (+Ca)
- ❑ Chicken (C) > Beef (B) OR beef/chicken with added calcium (+Ca) > without added calcium
- ❑ *(*)(*)(*) isomers

Results (3):

- What?
  - Comparison of digestion of beef *vs.* chicken
  - *+ vs.* with or without added calcium
  - 2 volunteers
    - P1 & P2
    - 3 biological replicates

  - Discriminative DNA adducts?

- How?
  - ❖ Sieve™:
    spectral data processing
  - ❖ Simca™:
    OPLS discriminant analysis

Most prominent findings

- Colonic microbiota
  - Active production/formation of (genotoxic) molecules

- DNA adduct profiling
  - Several DNA adduct types significantly higher or lower in different meat preparations…
    - …

- Discriminant analysis
    - …

Thank you!

www.vvv.ugent.be

www.PreProPed2016.be