FACULTY OF VETERINARY MEDICINE
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DEPARTMENT OF VETERINARY PUBLIC HEALTH AND FOOD SAFETY
LABORATORY OF CHEMICAL ANALYSIS

DNA ADDUCT MARKERS ASSOCIATED WITH THE GASTROINTESTINAL DIGESTION OF RED MEAT

L. Vanhaecke, C. Rombouts, T. Van Hecke, E. Vossen, S. De Smet & L.Y. Hemeryck

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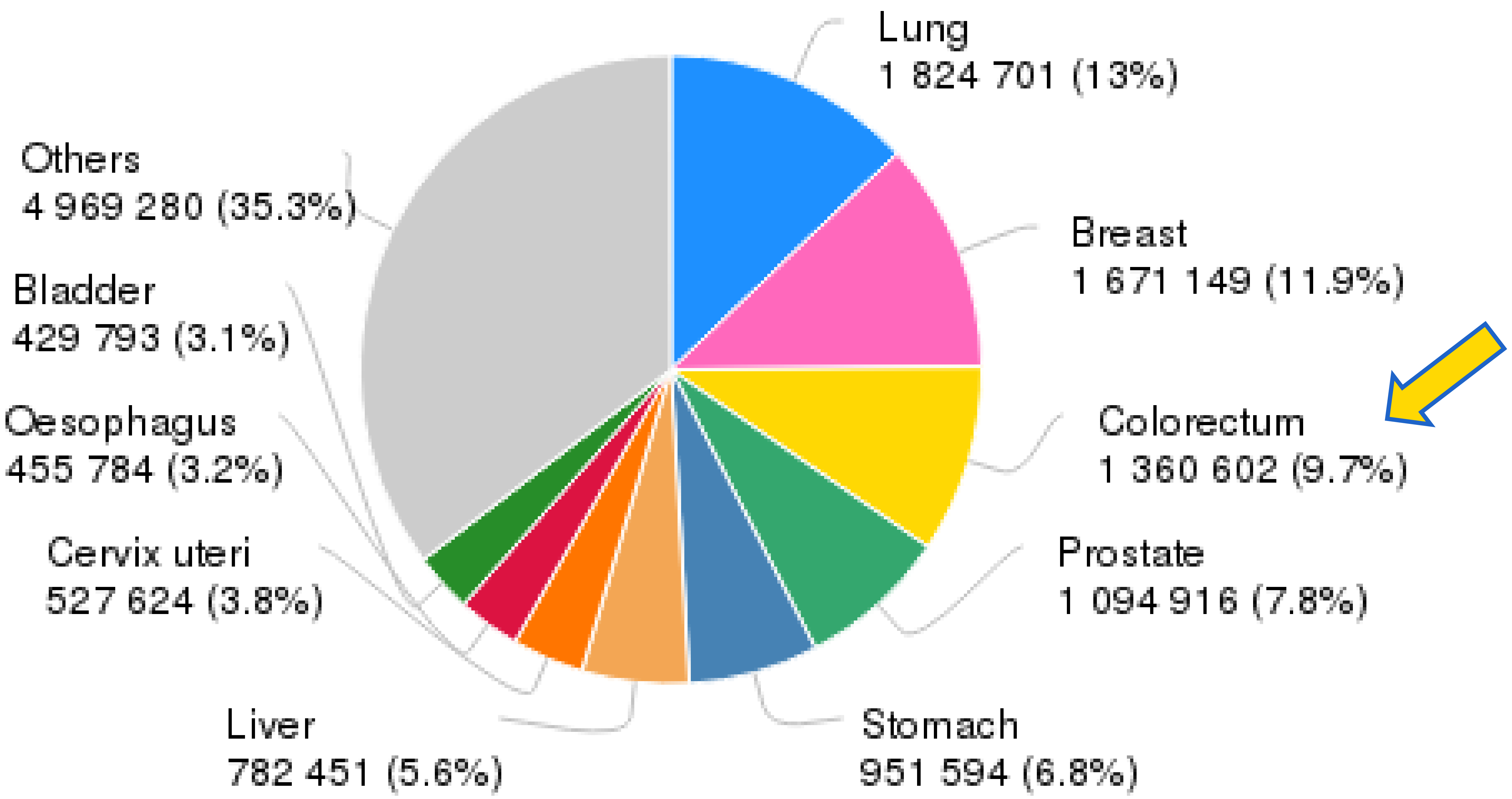
WORLDWIDE CANCER INCIDENCE

Estimated number of incidence cases, both sexes, worldwide (top 10 cancer sites) in 2012

World Health Organization



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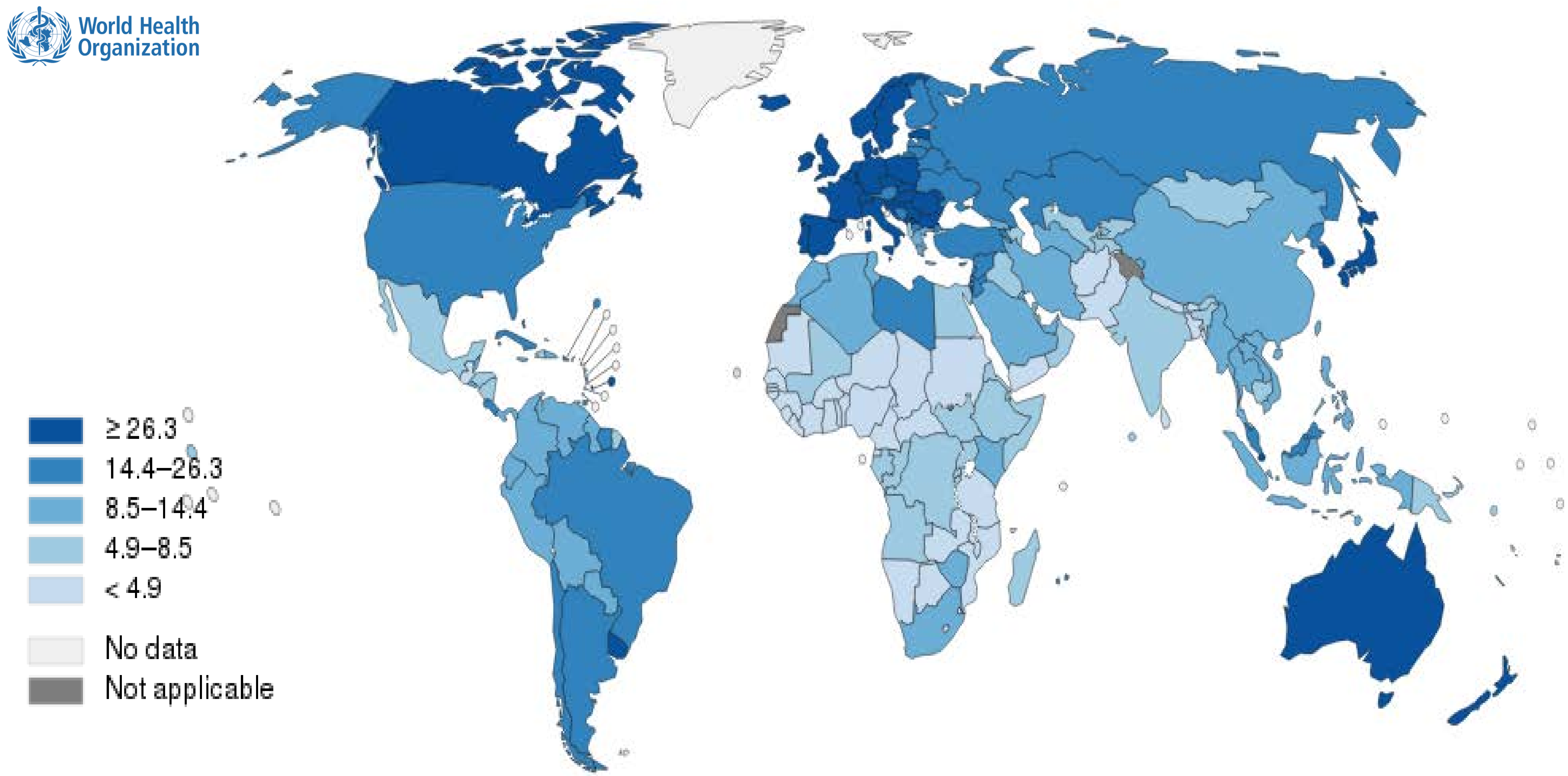
WORLDWIDE COLORECTAL CANCER INCIDENCE

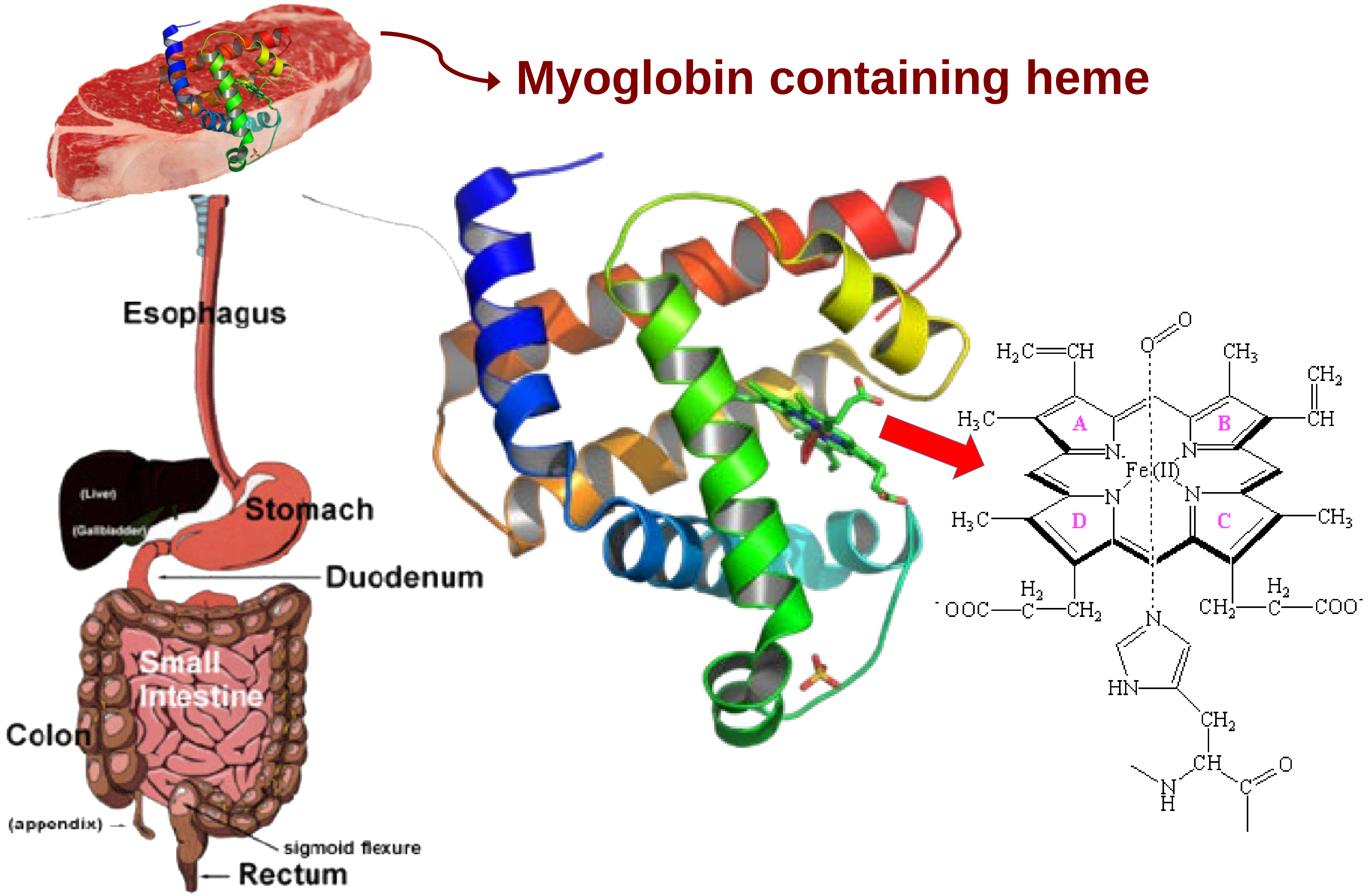
Estimated age-standardized rates (global) of incidence,
both sexes, colorectal cancer, worldwide in 2012



COLORECTAL CANCER (CRC) RISK

Factors that increase risk		Relative risk
	Alcohol consumption (heavy *vs.* nondrinkers)	1.6
	Obesity	1.2
	Red meat consumption	1.2
	Processed meat consumption	1.2
	Smoking (current *vs.* never)	1.2
Factors that decrease risk		**Relative risk**
	Physical activity	0.7
	Dairy consumption	0.8
	Fruit consumption	0.9
	Vegetable consumption	0.9
	Total dietary fiber (10 g/day)	0.9

RED *VS.* WHITE MEAT: HEME HYPOTHESIS



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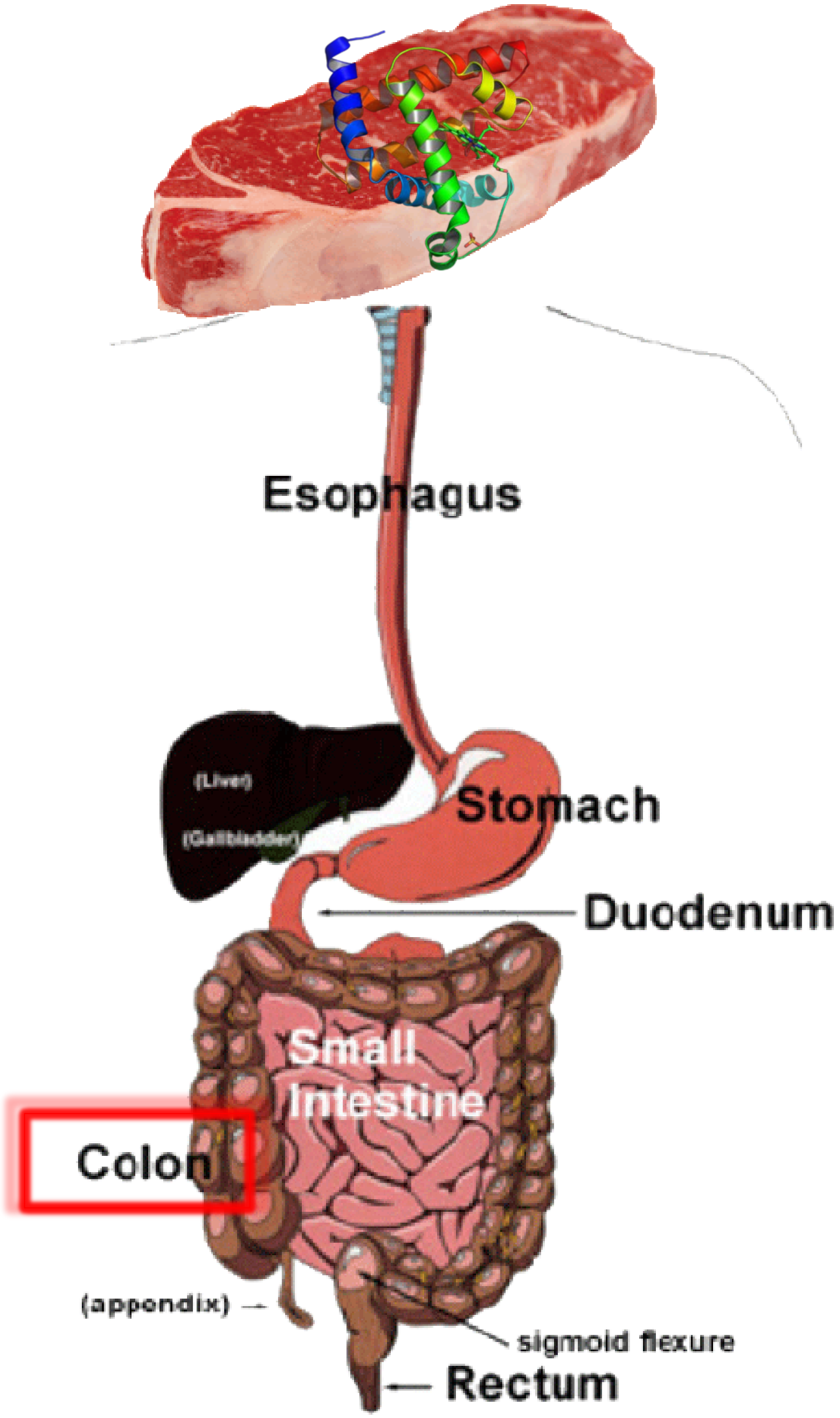
RED *VS.* WHITE MEAT: HEME HYPOTHESIS



- Passage through gastrointestinal tract
- Non-absorbed fraction

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

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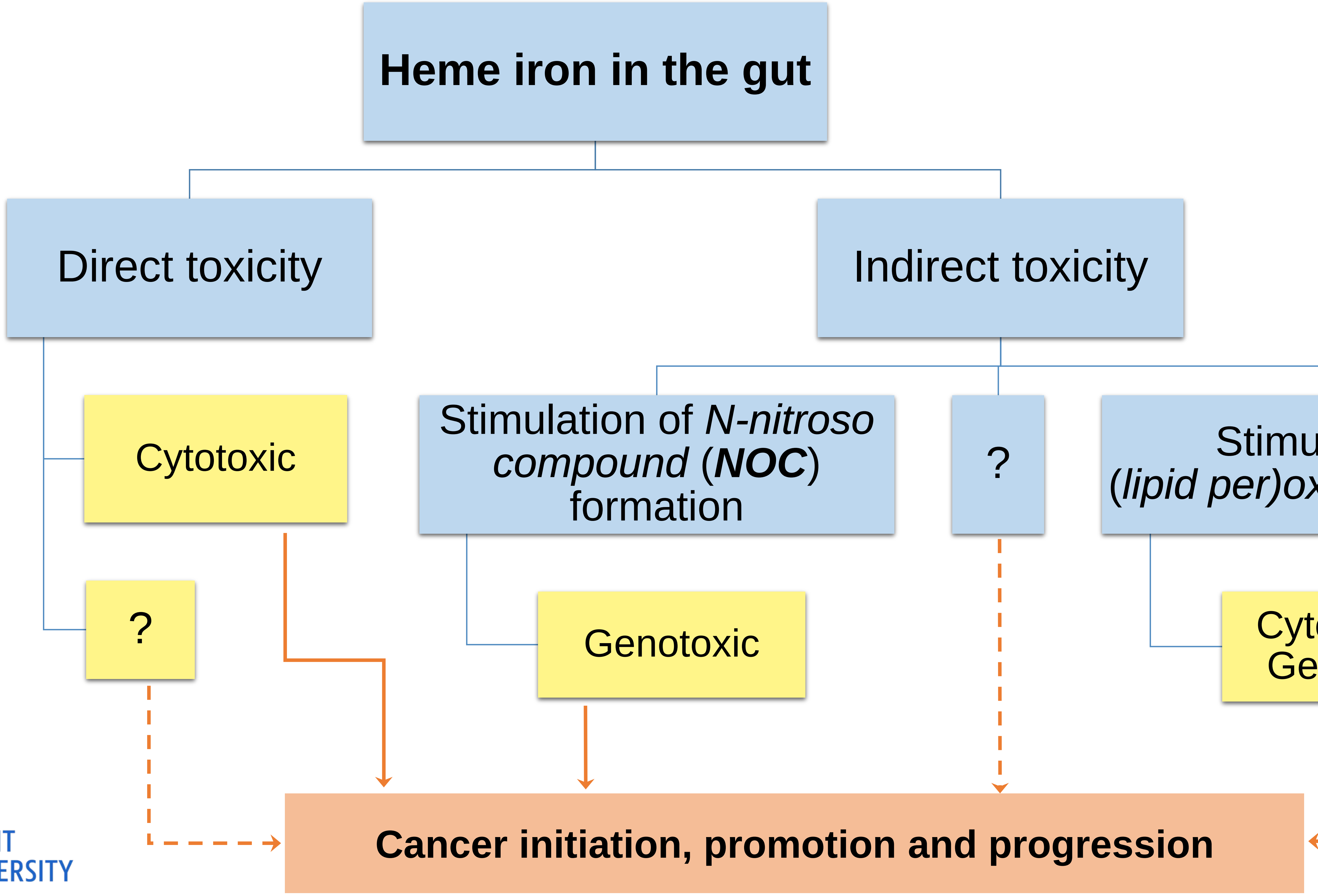
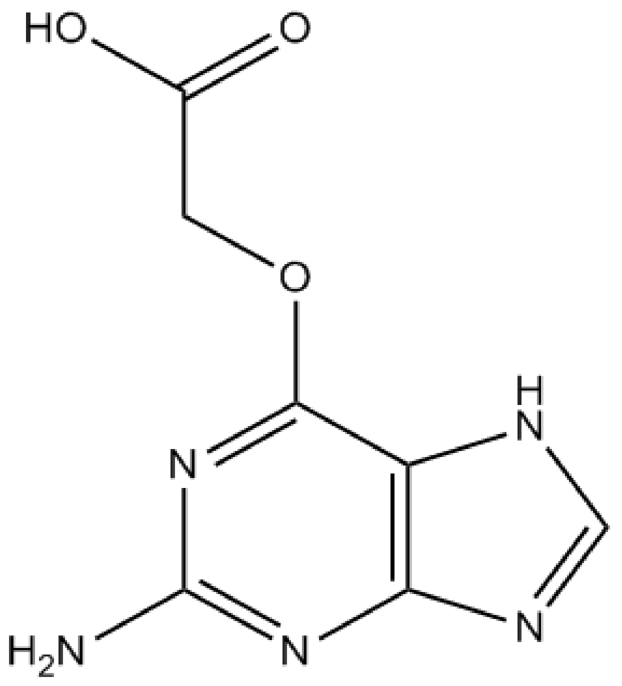
HEME IRON TOXICITY





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)

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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-Exactive^{TM})

- Successful validation:

  Hemeryck et al., 2015, *Analytica Chimica Acta*

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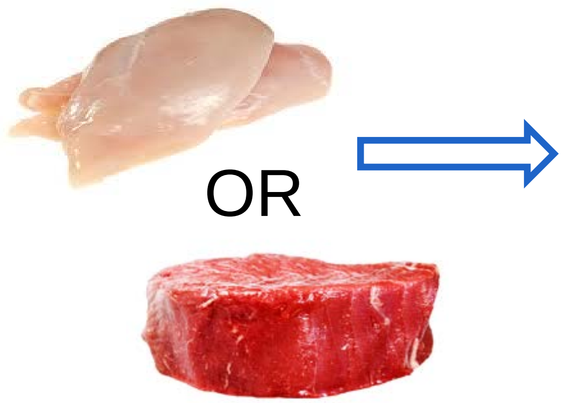
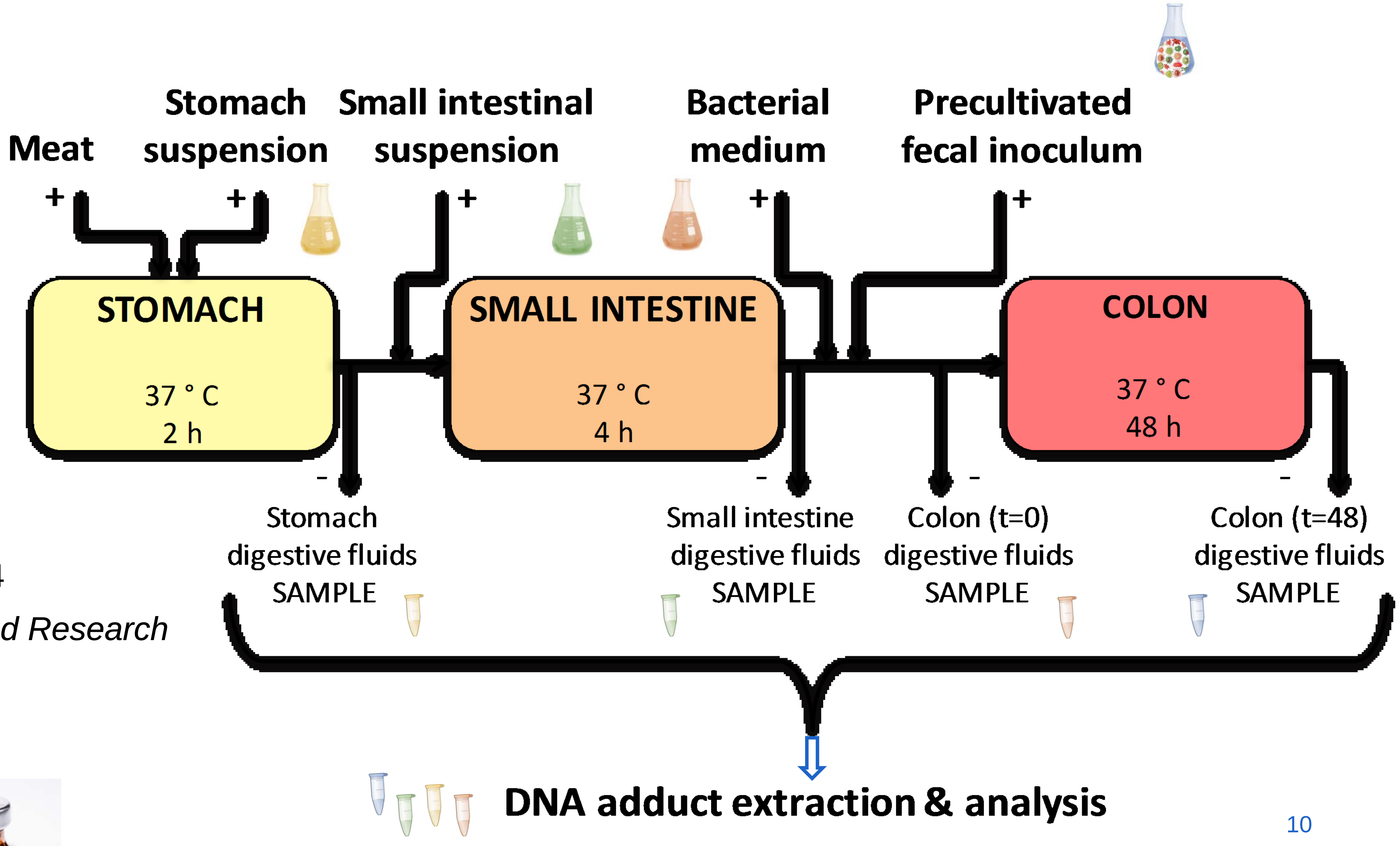
RED *VS.* WHITE MEAT (1): ***IN VITRO*** DIGESTION MODEL





Vanden Bussche et al. 2014

Molecular Nutrition and Food Research

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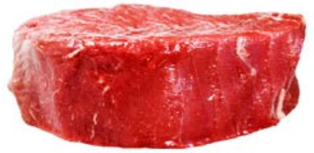
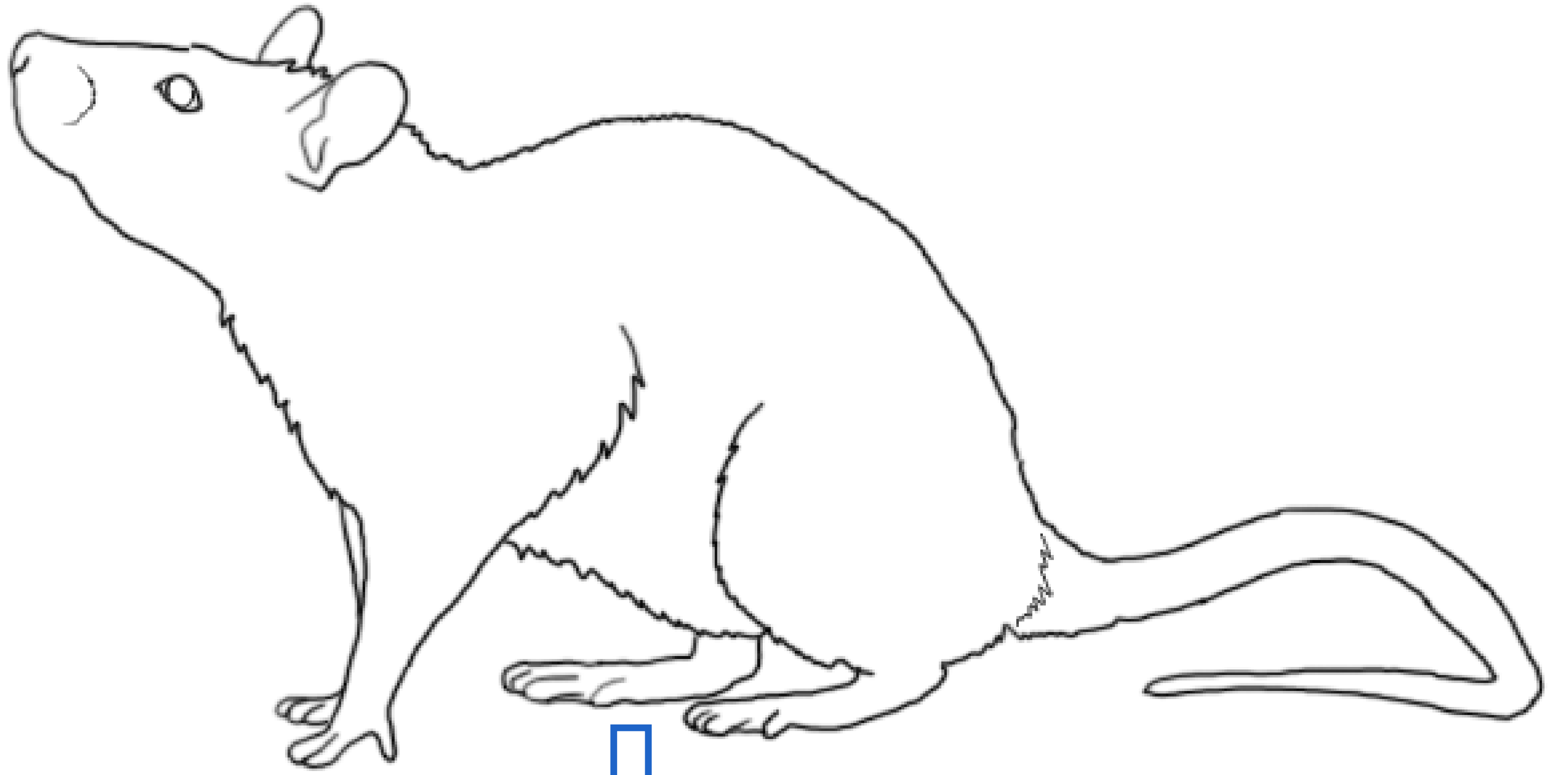
RED *VS.* WHITE MEAT (2): ***IN VIVO*** RAT MODEL

❖14-day feeding trial

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

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2016

In vitro DNA adduct profiling to mechanistically link red meat consumption to colon cancer promotion†

Lieselot Y. Hemeryck,[a] Caroline Rombouts,[a] Thomas Van Hecke,[b] Lieven Van Meulebroek,[a] Julie Vanden Bussche,[a] Stefaan De Smet[b] and Lynn Vanhaecke*[a]

Toxicology Research

ROYAL SOCIETY OF CHEMISTRY

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?

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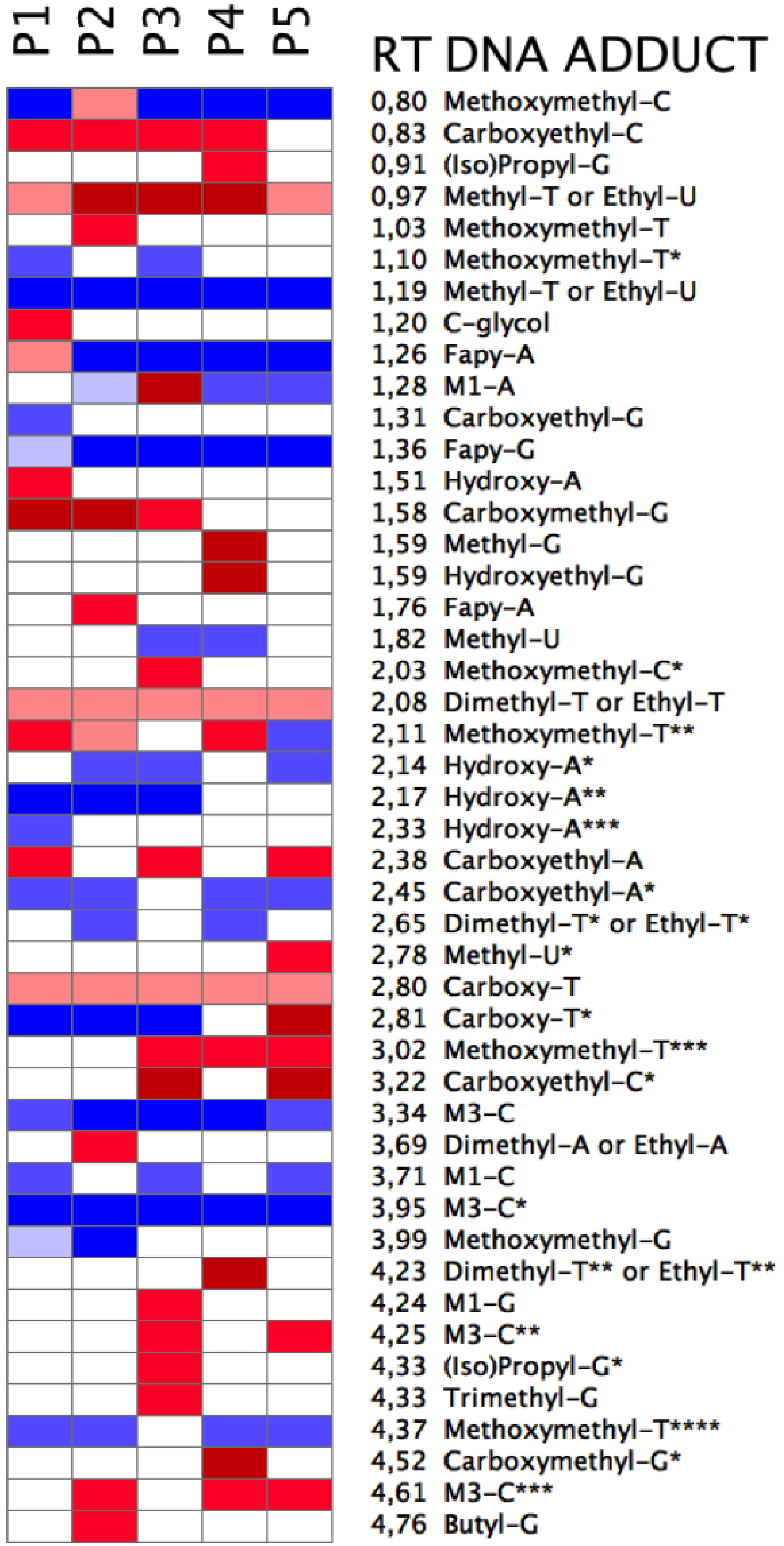
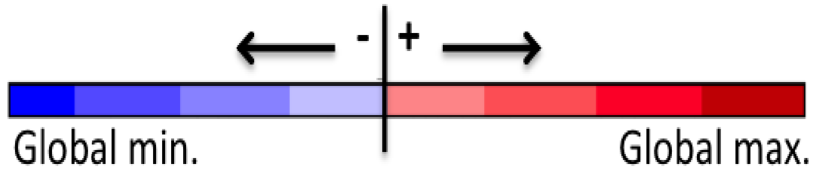
- DNA adduct formation upon the ***in vitro* digestion of beef** using 5 different fecal inocula: P1-P5
- Pre-colonic levels subtracted from post-colonic levels = representation of in- or decrease during colonic fermentation

➔ Interindividual variation

➔ Some DNA adduct types rise, whilst others decrease during colonic fermentation





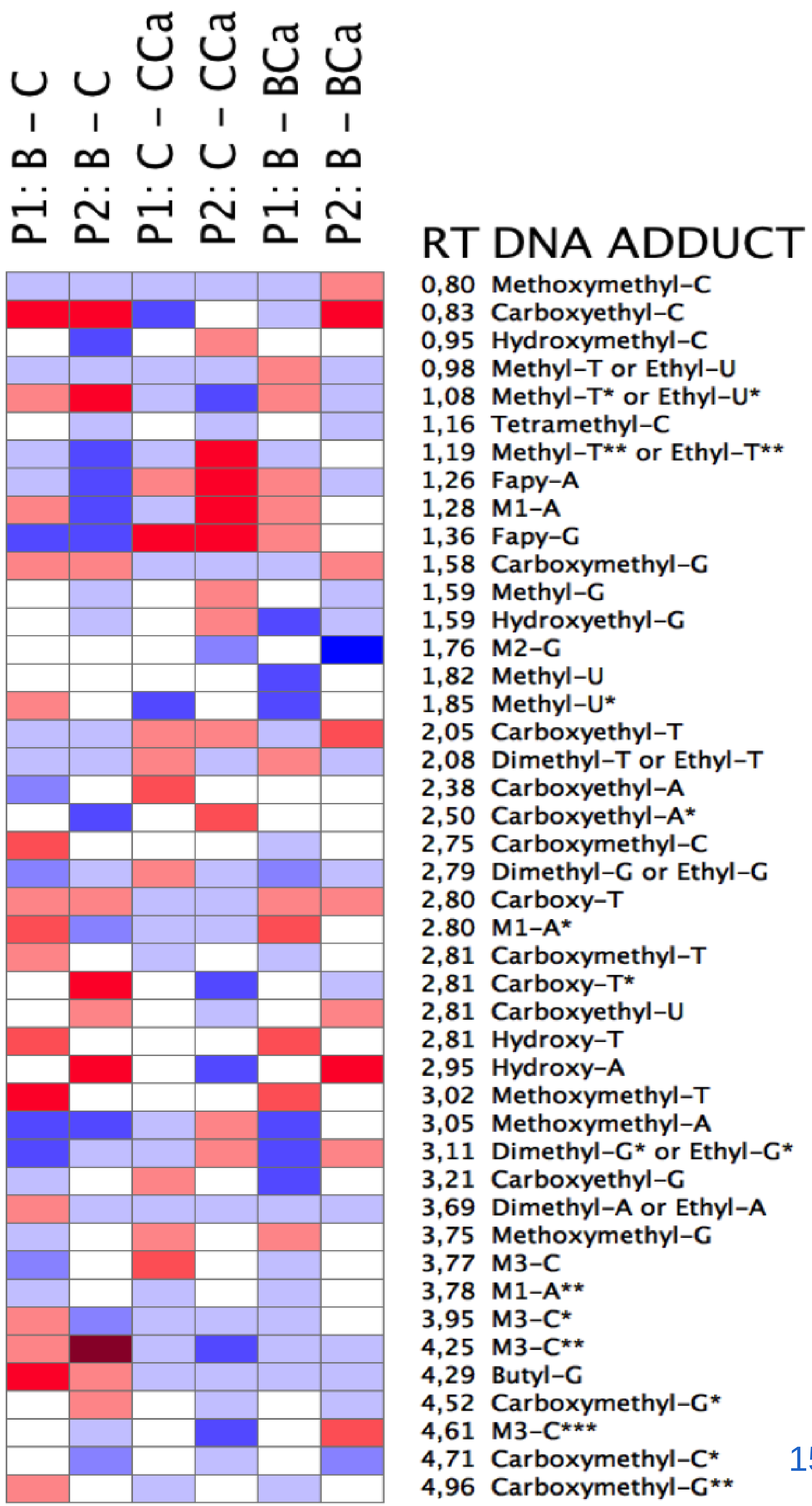
❖ DNA adduct formation upon the ***in vitro* digestion of different meat types** using 2 different fecal inocula: P1 & P2

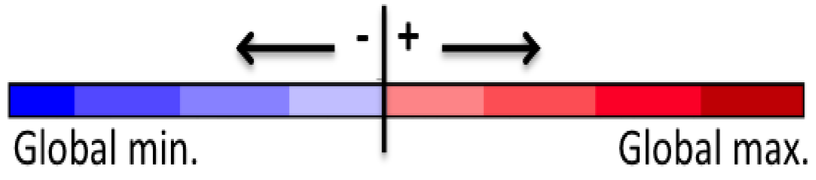
❖ Comparing:

- Beef *vs.* chicken
- Non-supplemented beef or chicken meat *vs.* beef or chicken supplemented with $CaCO_3$

❖ In (pre- and) post-colonic digestion samples

➔ Meat type strongly influences DNA adduct formation





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Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

2017

DNA adductomics to study the genotoxic effects of red meat consumption with and without added animal fat in rats

Lieselot Y. Hemeryck[a], Thomas Van Hecke[b], Els Vossen[b], Stefaan De Smet[b], Lynn Vanhaecke[a,*]

- *In vivo* digestion of beef or chicken by Sprague-Dawley rats

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

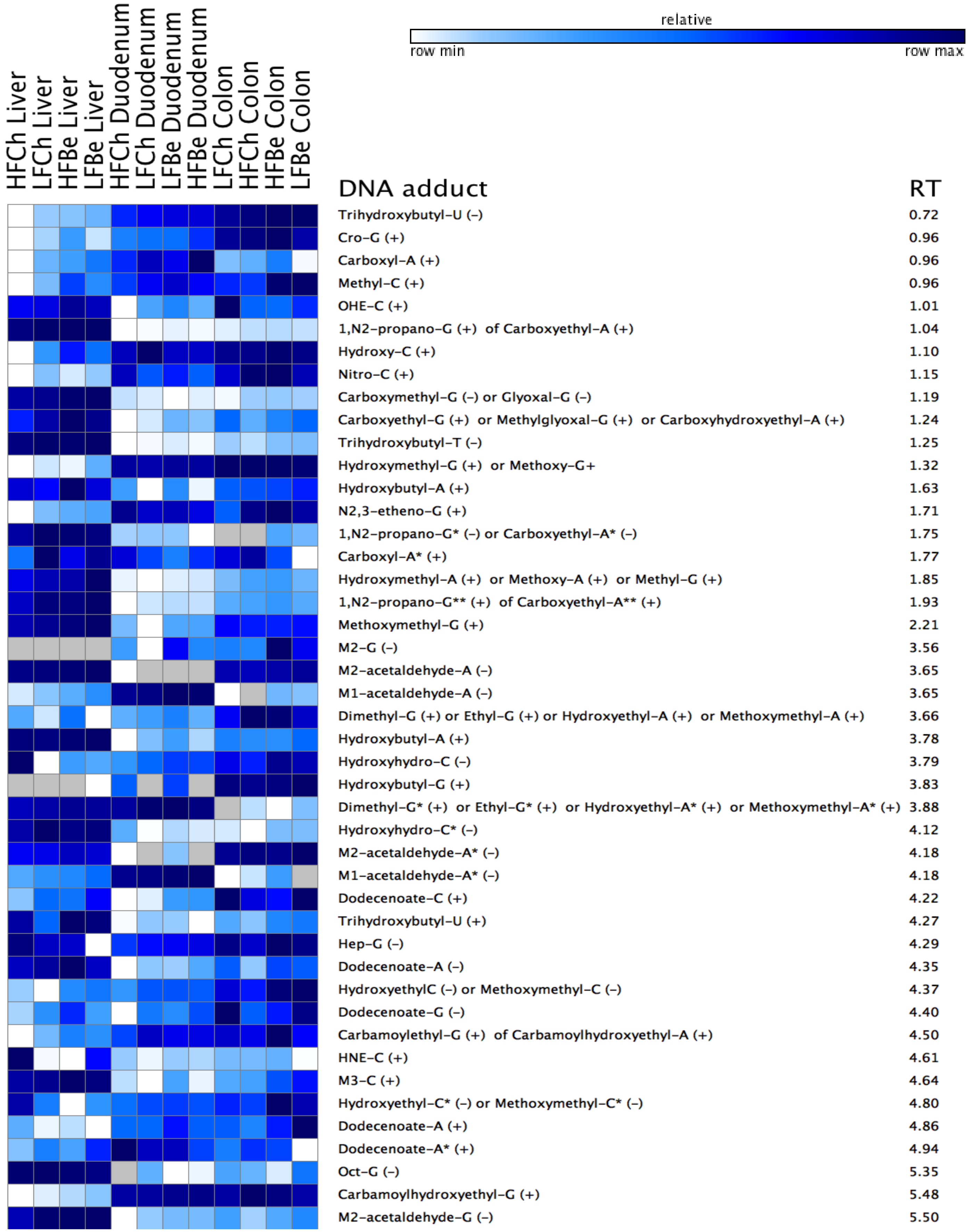
+ Investigation of the interfering role of dietary fat

- ❖ 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



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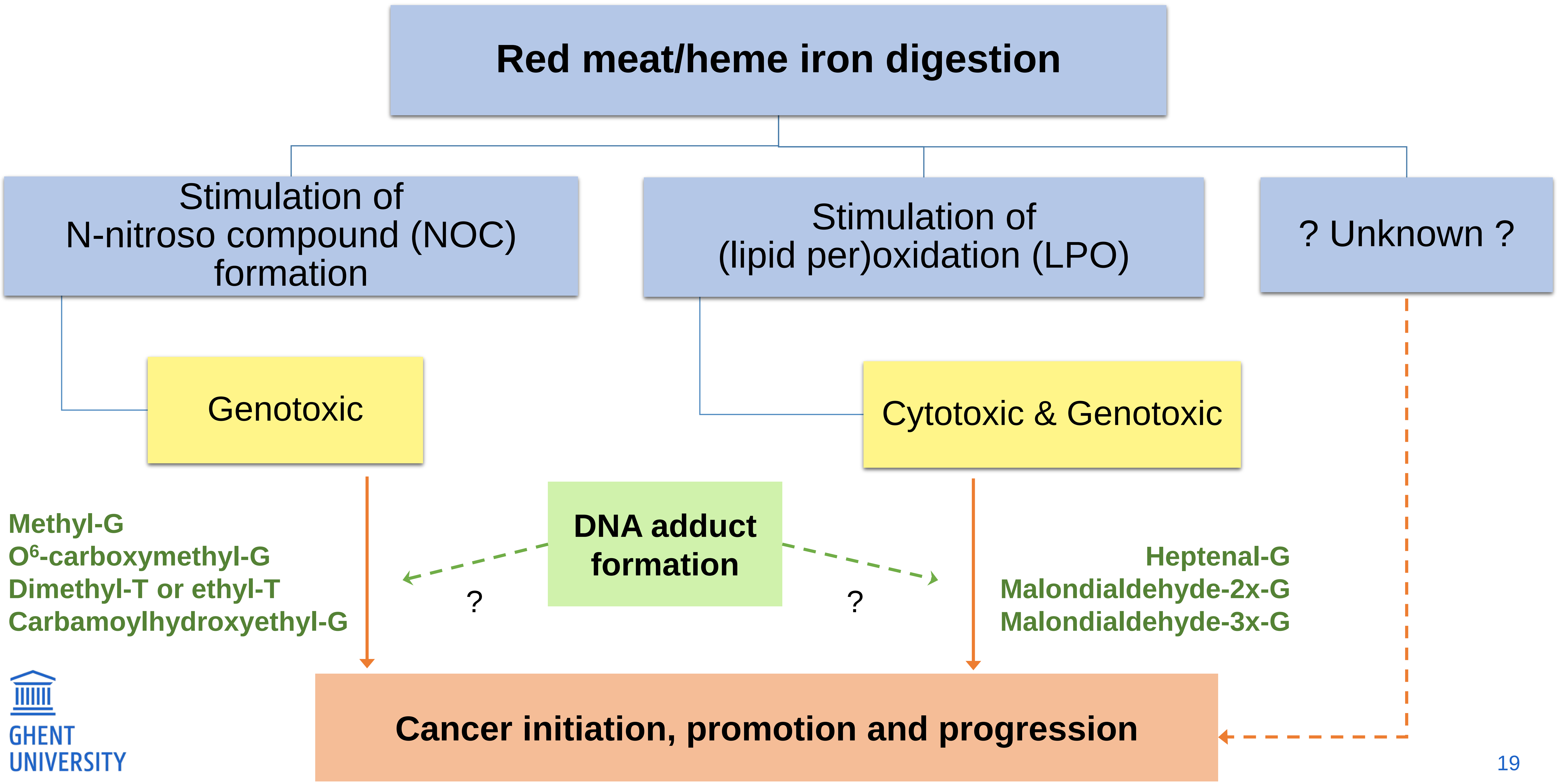
DNA ADDUCTS WITH RED MEAT MARKER POTENTIAL

DNA adduct name	DNA adduct type	Context	Test	p-value or VIP score
O^6-Carboxymethyl-G	DNA alkylation	*In vitro* (x3)	ANOVA & t-test	p = 0.05, p < 0.01, p = 0.05
Dimethyl-T or ethyl-T	DNA alkylation	*In vitro* (x2)	Sieve™ pairwise comparison & Simca™ analysis	p = 0.02, VIP = 1.95
Methyl-G	DNA alkylation	*In vitro* (x2)	Simca™ analysis & t-test	VIP = 1.23, p = 0.03
Malondialdehyde-2x-G	Lipid peroxidation & attack of DNA	*In vitro* & *in vivo*	Sieve™ pairwise comparison & GENE-E marker selection	p = 0.05, p = 0.02
Heptenal-G	Lipid peroxidation & attack of DNA	*In vitro* & *in vivo*	t-test	p = 0.05, p = 0.03
Carbamoylhydroxyethyl-G	DNA alkylation	*In vitro* & *in vivo*	t-test	p = 0.03, p = 0.04
Malondialdehyde-3x-C	Lipid peroxidation & attack of DNA	*In vitro* (x2)	Sieve™ pairwise comparison & t-test	p < 0.01, p = 0.01

CONCLUSIONS: RELEVANT TO RED MEAT-CRC LINK?



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PARALLEL RESEARCH: HRMS BASED ‘GUT’ METABOLOMICS

analytical chemistry

Article

pubs.acs.org/ac

Validated High Resolution Mass Spectrometry-Based Approach for Metabolomic Fingerprinting of the Human Gut Phenotype

Julie Vanden Bussche,[*,†] Massimo Marzorati,[‡] Debby Laukens,[⊥] and Lynn Vanhaecke[†]

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2015

Holistic Lipidomics of the Human Gut Phenotype using Validated Ultra-High Performance Liquid Chromatography coupled to Hybrid Orbitrap Mass Spectrometry
Van Meulebroek et al., submitted (see also poster 22)

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

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PARALLEL RESEARCH: METABOLOMICS RED *VS.* WHITE MEAT

SCIENTIFIC REPORTS

OPEN

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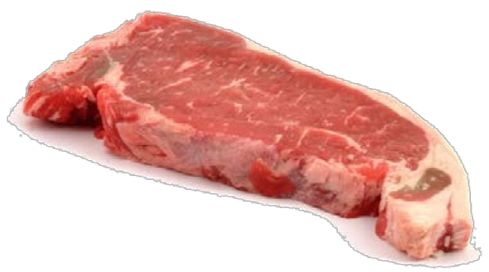
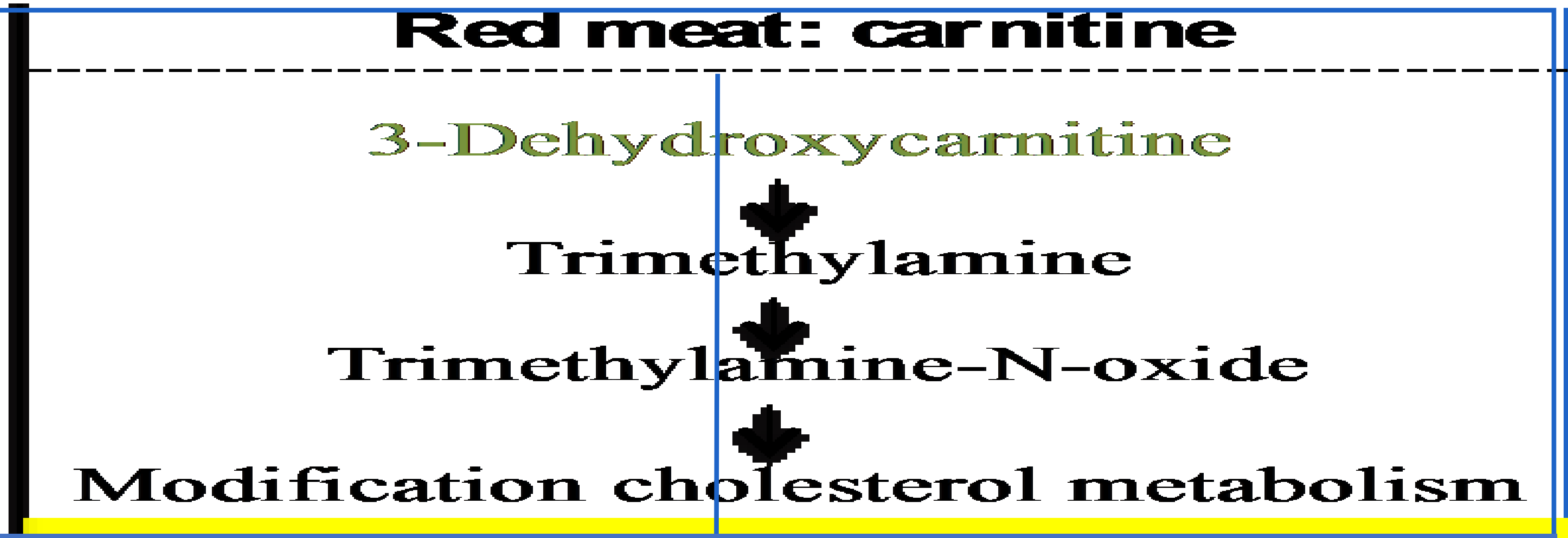


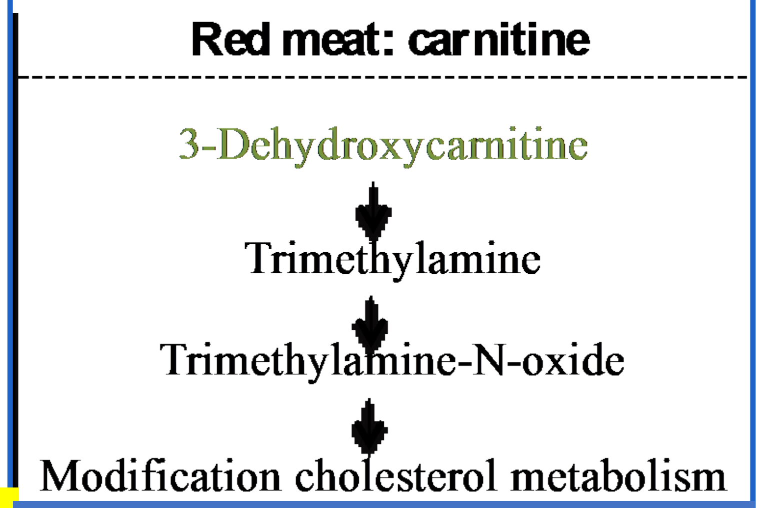
Discovery of **5 discriminating metabolites** with potential involvement red meat related diseases

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



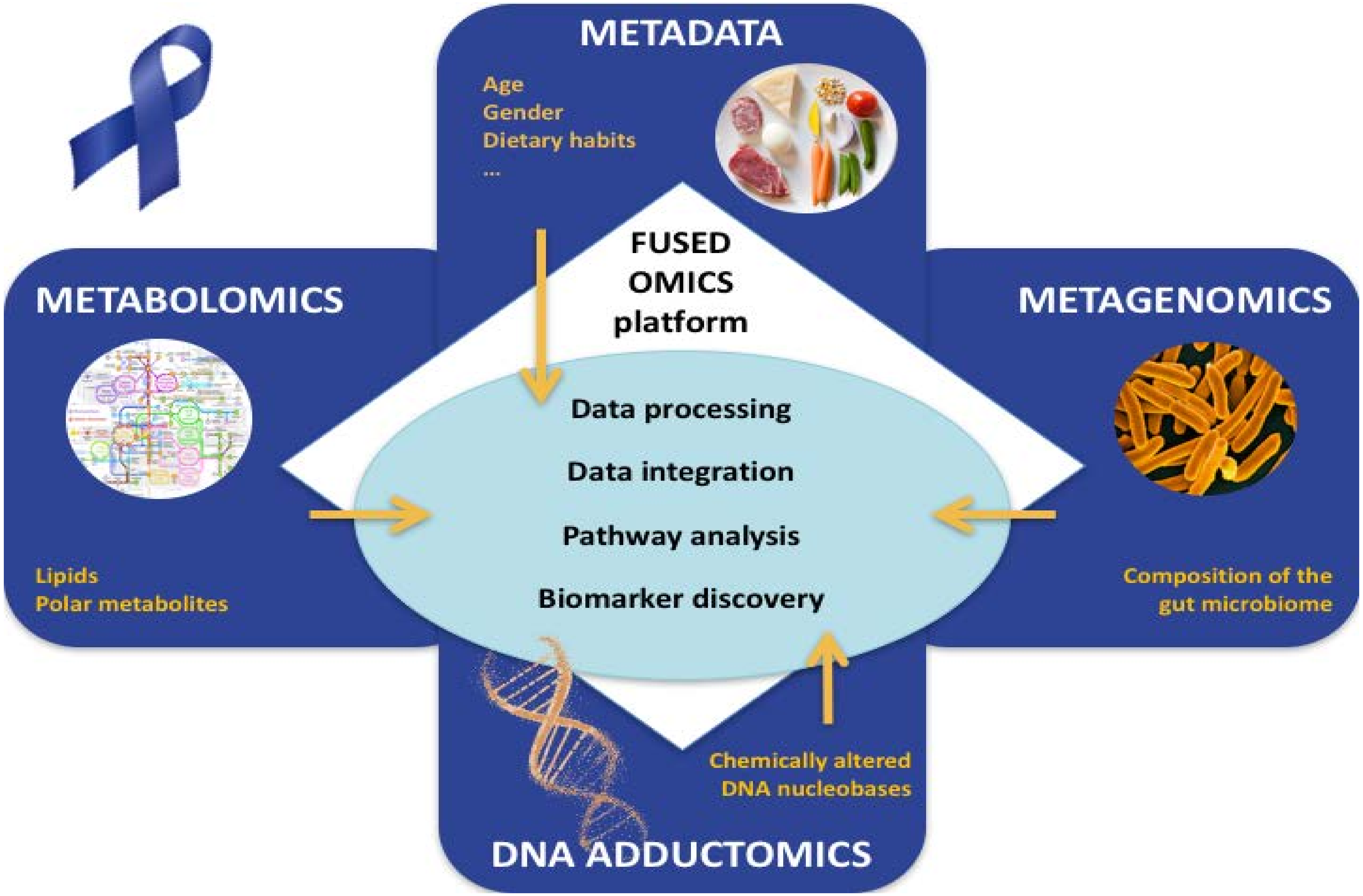
Initiation, promotion and progression of cancer

Progression cancer, diabetes mellitus



Cardiovascular disease

FUTURE RESEARCH: FUSED OMICS



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THANK YOU!

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