



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

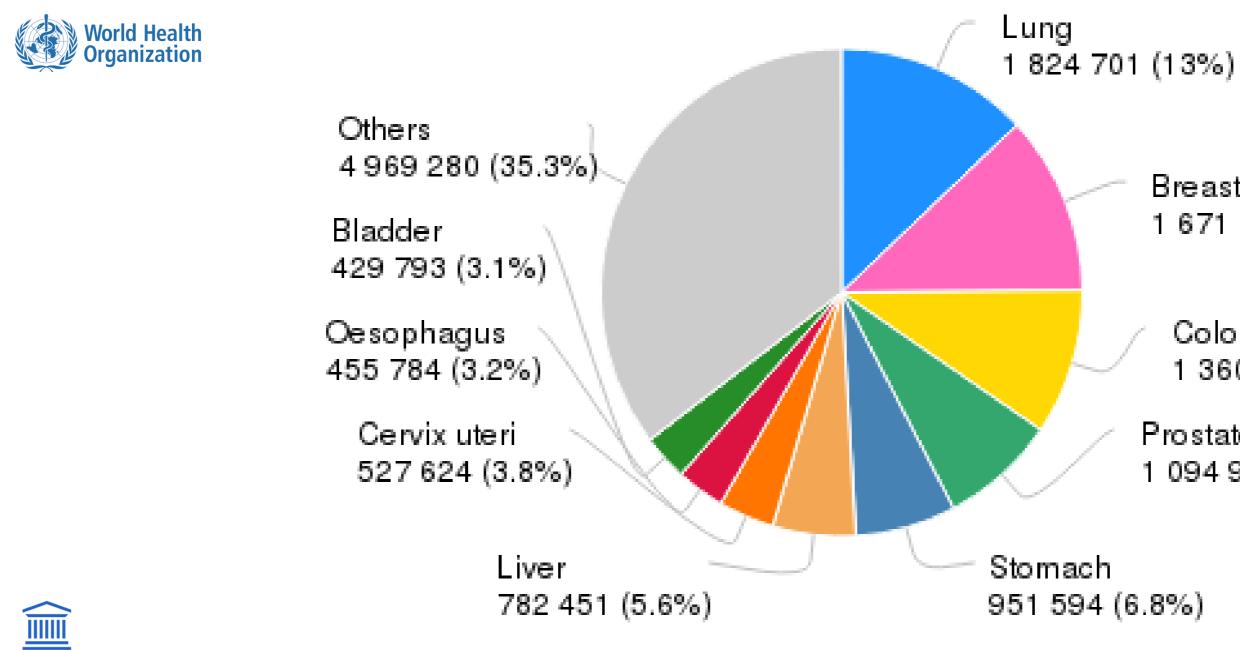


DEPARTMENT OF VETERINARY PUBLIC HEALTH AND FOOD SAFETY



WORLDWIDE CANCER INCIDENCE

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



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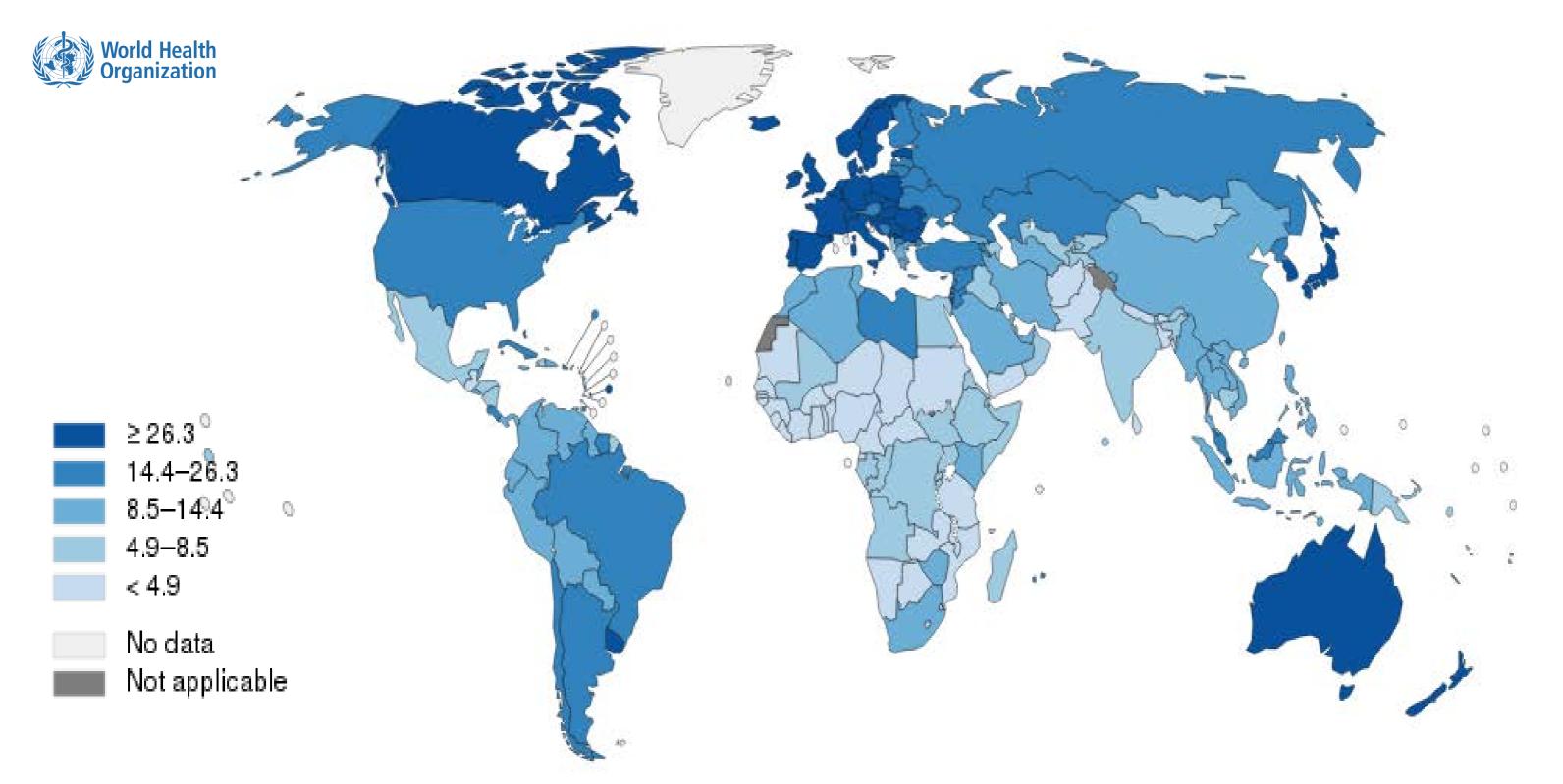
- Breast 1 671 149 (11.9%)



- Colorectum 1 360 602 (9.7%)
- Prostate
- 1 094 916 (7.8%)

WORLDWIDE COLORECTAL CANCER INCIDENCE

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

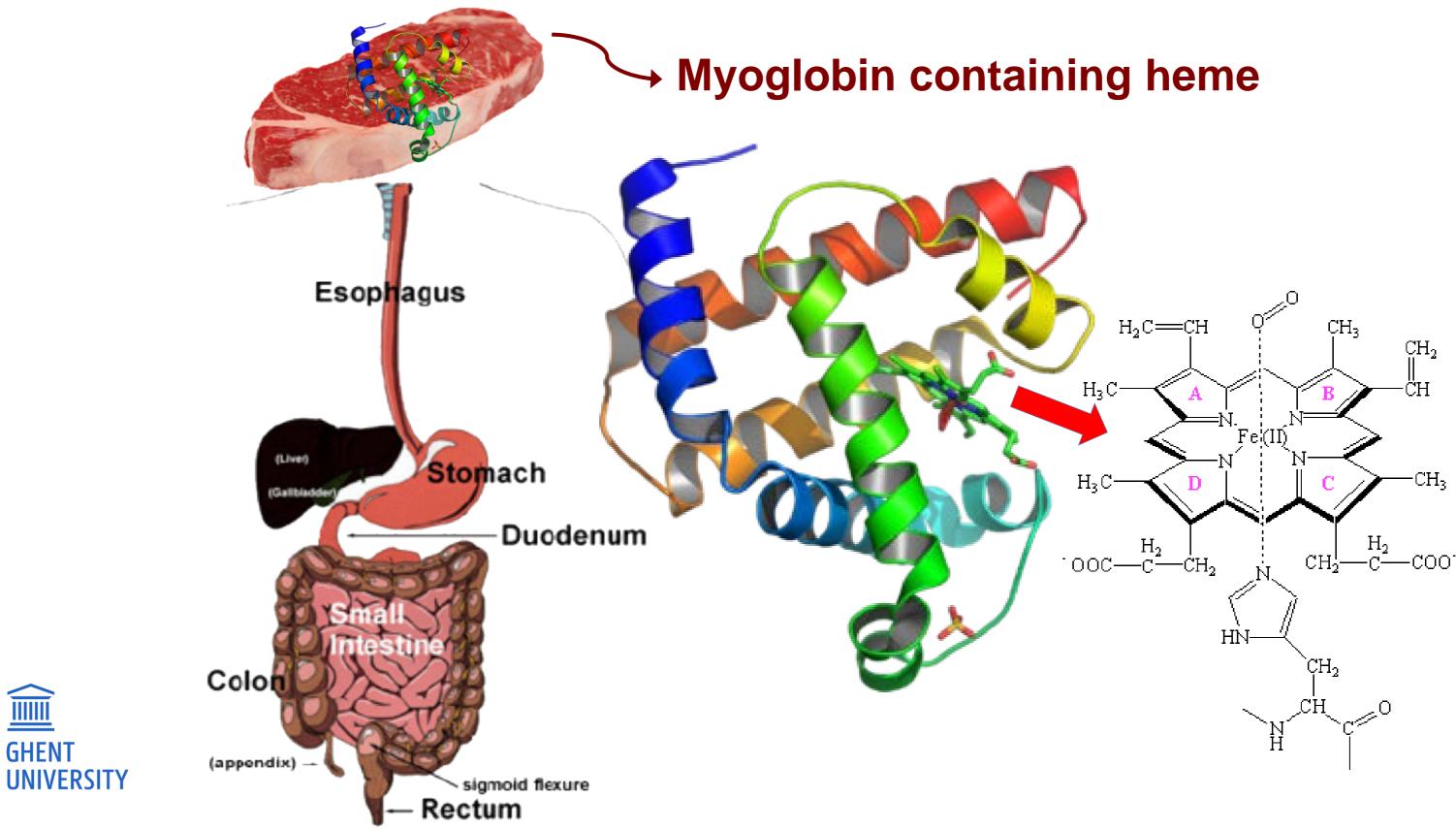


COLORECTAL CANCER (CRC) RISK

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		Deletive riel
		Relative risk
	Physical activity	0.7
	Physical activity Dairy consumption	
		0.7
	Dairy consumption	0.7 0.8

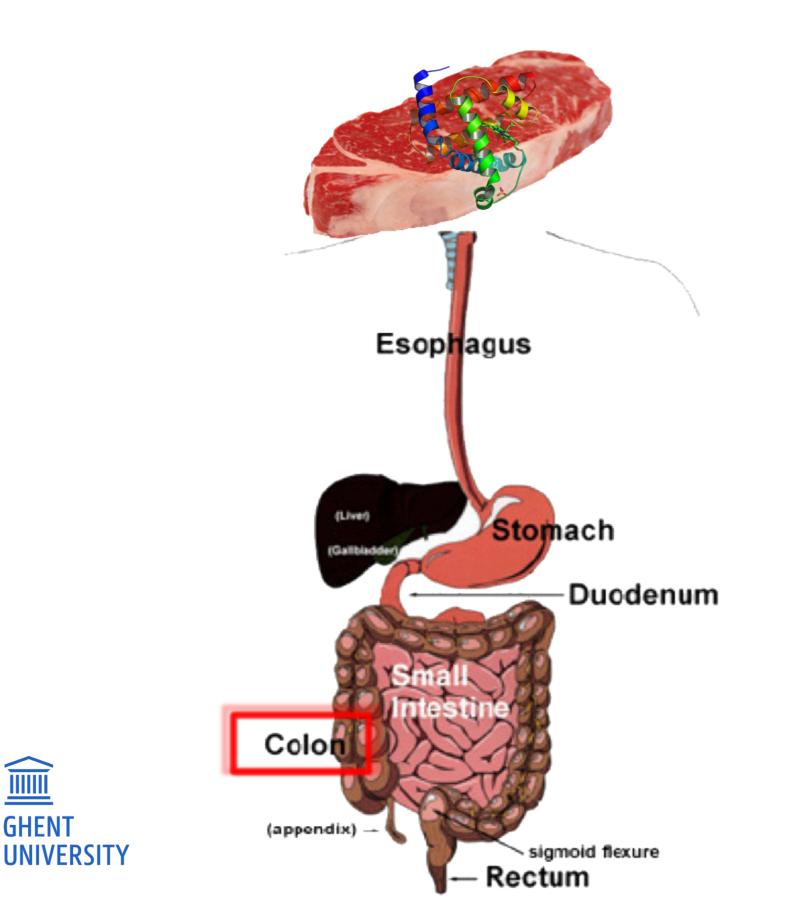
RED VS. WHITE MEAT: HEME HYPOTHESIS

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<u>RED VS. WHITE MEAT: HEME HYPOTHESIS</u>



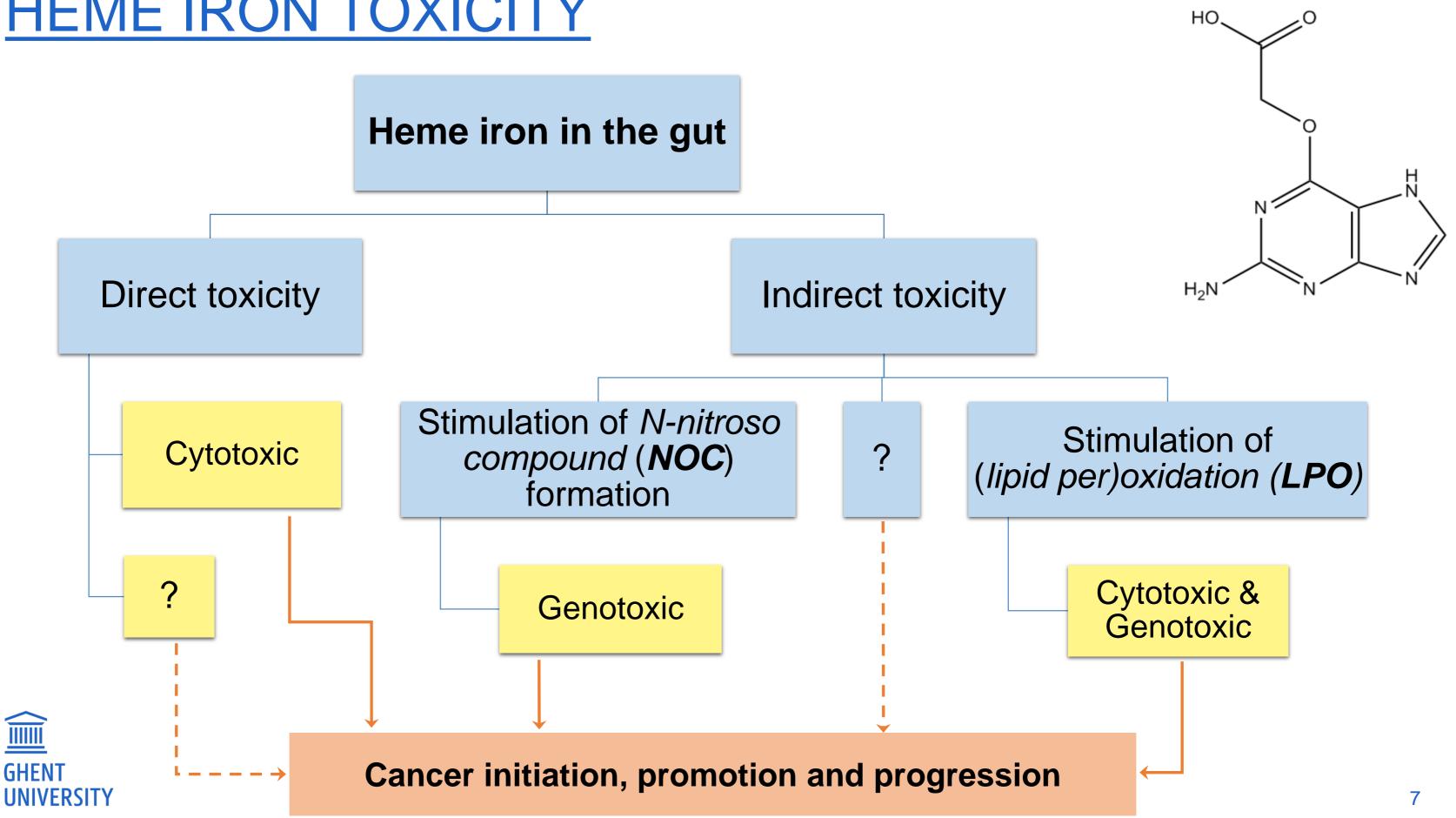
- Passage through gastrointestinal tract Non-absorbed fraction

transformations



 \rightarrow Passage through ascending, transverse and descending **colon**: catalyzes a number of **endogenous**

HEME IRON TOXICITY



STUDY GOALS

1. Install a UHPLC-HRMS based DNA adductomics methodology

 \rightarrow To facilitate targeted as well as untargeted DNA adduct analysis

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



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[™])

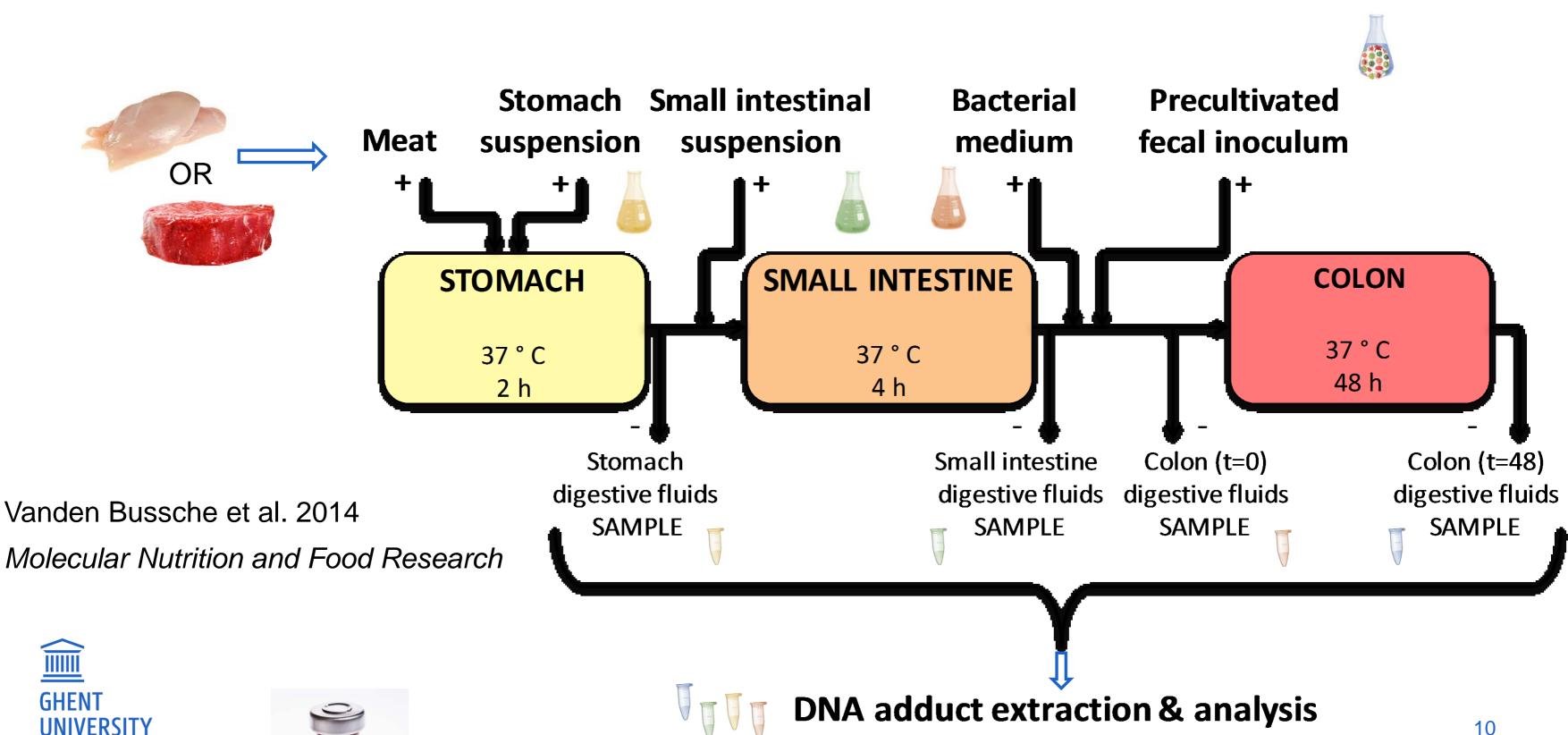
Successful validation:

Hemeryck et al., 2015, Analytica Chimica Acta



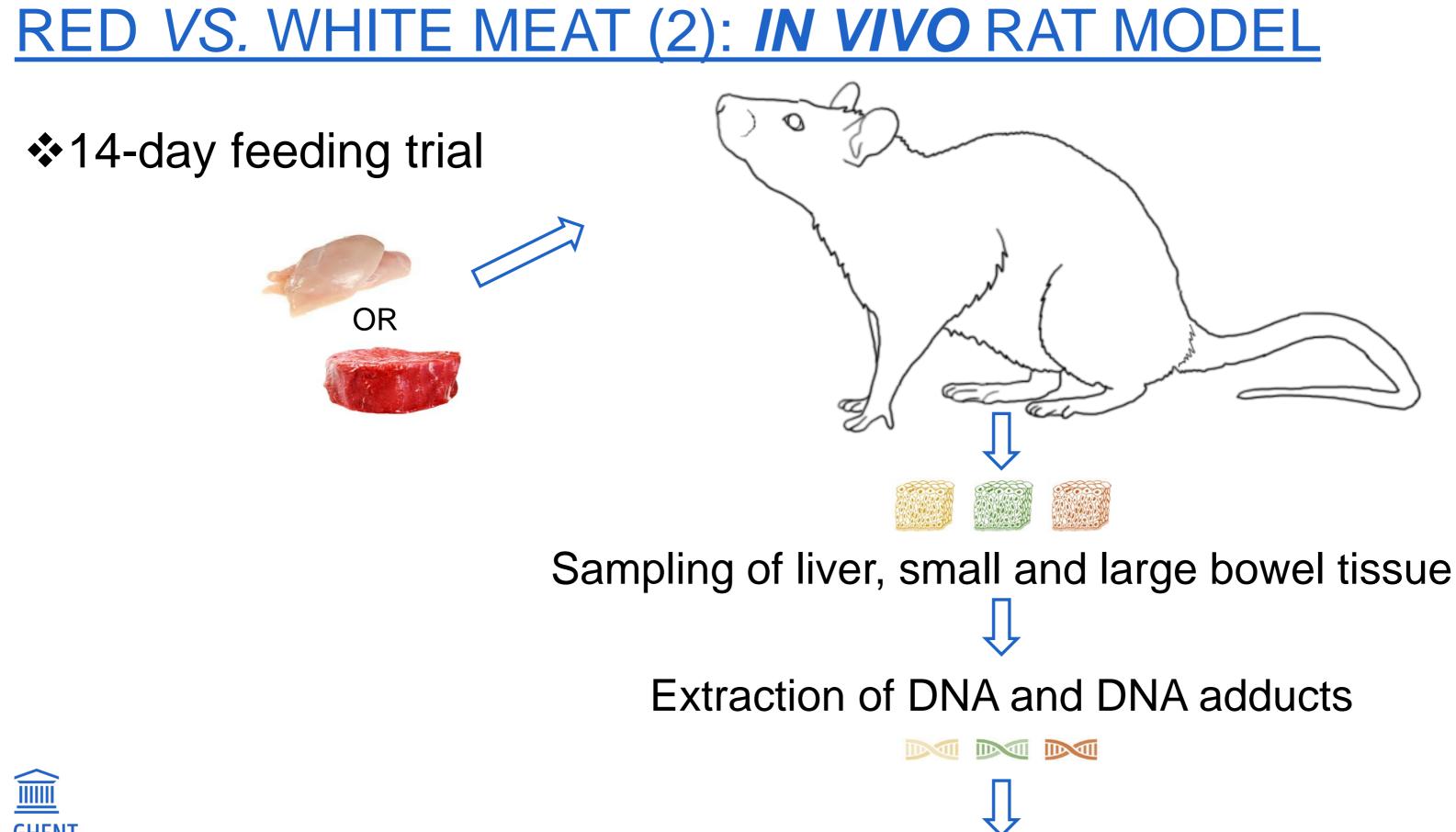


RED VS. WHITE MEAT (1): IN VITRO DIGESTION MODEL











DNA adduct analysis

CONDUCTED EXPERIMENTS & STUDIES

- In vitro digestion of chicken, pork & 3. In vitro digestion of chicken & beef
 10 fecal inocula
 - 15 fecal inocula

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 Limited to targeted DNA adduct analysis

- Targeted & untargeted DNA adduct analysis
- Additionally: assessment of effect of myoglobin addition

2.	In vitro digestion of chicken & beef 4.	<u>In vivo dig</u>
	 5 fecal inocula 	 14-day fe
	 Targeted & untargeted DNA adduct 	Sprague
	analysis	 Targeted
	 Additionally: assessment of effect of 	analysis
	calcium (CaCO ₃) addition	Additiona
		lard cont

- igestion of chicken & beef
- feeding trial
- e-Dawley rats
- d & untargeted DNA adduct
- ally: assessment of effect of tent



Cite this: DOI: 10.1039/c6tx00079g

2016

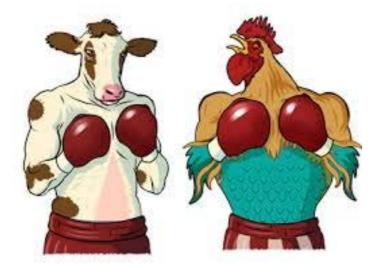
In vitro DNA adduct profiling to mechanistically link red meat consumption to colon cancer promotion[†]

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

1.1 *In vitro* digestion of beef using 5 different fecal inocula

1.2 Selection of 2 fecal inocula for further investigation: Beef vs. Chicken $CaCO_3$ supplementation &





→ DNA adduct formation?

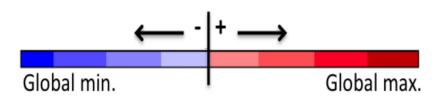
Toxicology Research

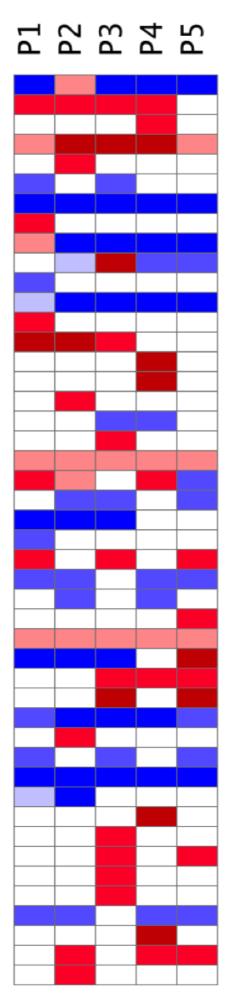


→ DNA adduct formation?

- 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





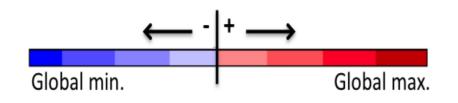


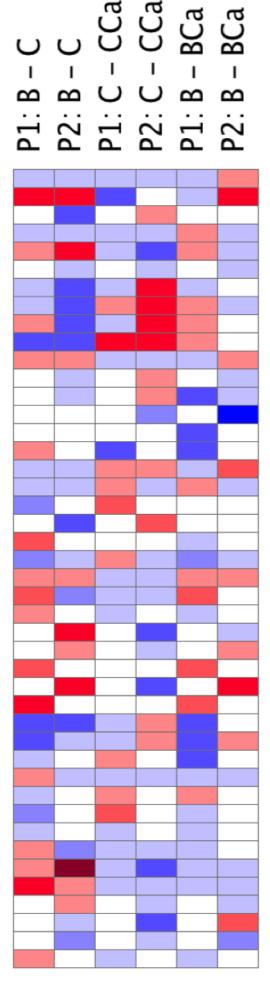
RT DNA ADDUCT

- 0,80 Methoxymethyl-C
- 0,83 Carboxyethyl-C
- 0,91 (Iso)Propyl-G
- 0,97 Methyl-T or Ethyl-U
- 1,03 Methoxymethyl-T
- 1,10 Methoxymethyl-T*
- 1,19 Methyl-T or Ethyl-U
- 1,20 C-glycol
- 1,26 Fapy-A
- 1,28 M1-A
- 1,31 Carboxyethyl-G
- 1,36 Fapy-G
- 1,51 Hydroxy-A
- 1,58 Carboxymethyl-G
- 1,59 Methyl-G
- 1,59 Hydroxyethyl-G
- 1,76 Fapy-A
- 1,82 Methyl-U
- 2,03 Methoxymethyl-C*
- 2,08 Dimethyl-T or Ethyl-T
- 2,11 Methoxymethyl-T**
- 2,14 Hydroxy-A*
- 2,17 Hydroxy-A**
- 2,33 Hydroxy-A***
- 2,38 Carboxyethyl-A
- 2,45 Carboxyethyl-A*
- 2,65 Dimethyl-T* or Ethyl-T*
- 2,78 Methyl-U*
- 2,80 Carboxy-T
- 2,81 Carboxy-T*
- 3,02 Methoxymethyl-T***
- 3,22 Carboxyethyl-C*
- 3,34 M3-C
- 3,69 Dimethyl-A or Ethyl-A
- 3,71 M1-C
- 3,95 M3-C*
- 3,99 Methoxymethyl-G
- 4,23 Dimethyl-T** or Ethyl-T**
- 4,24 M1-G
- 4,25 M3-C**
- 4,33 (Iso)Propyl-G*
- 4,33 Trimethyl-G
- 4,37 Methoxymethyl-T****
- 4,52 Carboxymethyl-G*
- 4,61 M3-C***
- 4,76 Butyl-G

- 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







RT DNA ADDUCT

- 0,80 Methoxymethyl-C
- 0,83 Carboxyethyl-C
- 0,95 Hydroxymethyl-C
- 0,98 Methyl-T or Ethyl-U 1,08 Methyl-T* or Ethyl-U*
- 1,16 Tetramethyl-C
- 1,19 Methyl-T** or Ethyl-T**
- 1,26 Fapy-A
- 1,28 M1-A
- 1,36 Fapy-G
- 1,58 Carboxymethyl-G
- 1,59 Methyl-G
- 1,59 Hydroxyethyl-G
- 1,76 M2-G
- 1,82 Methyl-U
- 1,85 Methyl-U*
- 2,05 Carboxyethyl-T
- 2,08 Dimethyl-T or Ethyl-T
- 2,38 Carboxyethyl-A
- 2,50 Carboxyethyl-A*
- 2,75 Carboxymethyl-C
- 2,79 Dimethyl-G or Ethyl-G
- 2,80 Carboxy-T
- 2.80 M1-A*
- 2,81 Carboxymethyl-T
- 2,81 Carboxy-T*
- 2,81 Carboxyethyl-U
- 2,81 Hydroxy-T
- 2,95 Hydroxy-A
- 3,02 Methoxymethyl-T
- 3,05 Methoxymethyl-A
- 3,11 Dimethyl–G* or Ethyl–G*
- 3,21 Carboxyethyl-G
- 3,69 Dimethyl-A or Ethyl-A
- 3,75 Methoxymethyl-G
- 3,77 M3-C
- 3,78 M1-A**
- 3,95 M3-C*
- 4,25 M3-C**
- 4,29 Butyl-G
- 4,52 Carboxymethyl-G*
- 4,61 M3-C***
- 4,71 Carboxymethyl-C*

15

4,96 Carboxymethyl-G**



Contents lists available at ScienceDirect

Food Chemistry

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

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 GHENT UNIVERSITY

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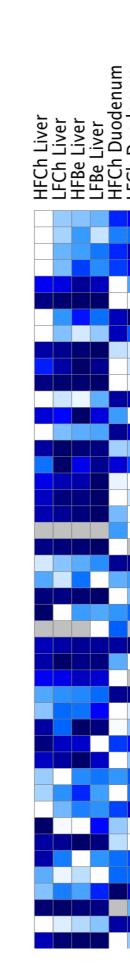






- DNA adduct formation in liver, duodenum & colon upon digestion of:
 - a low fat beef diet ('LFBe'), or
 - a low fat chicken diet ('LFCh'), or
 - a high fat beef diet ('HFBe'), or
 - a high fat chicken diet ('HFCh')
- ➔ Prominent difference according to tissue type
- ➔ Difference according to diet
- → 22 DNA adduct types increased due to beef and/or lard digestion







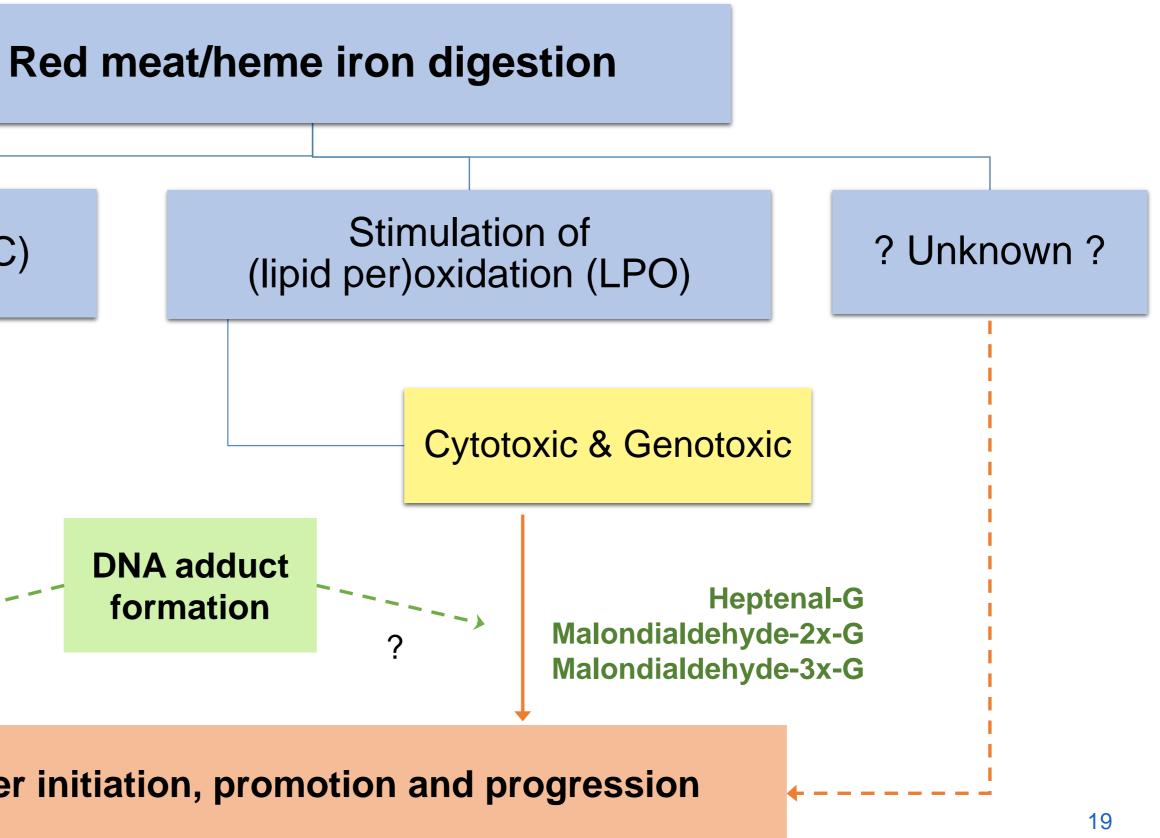
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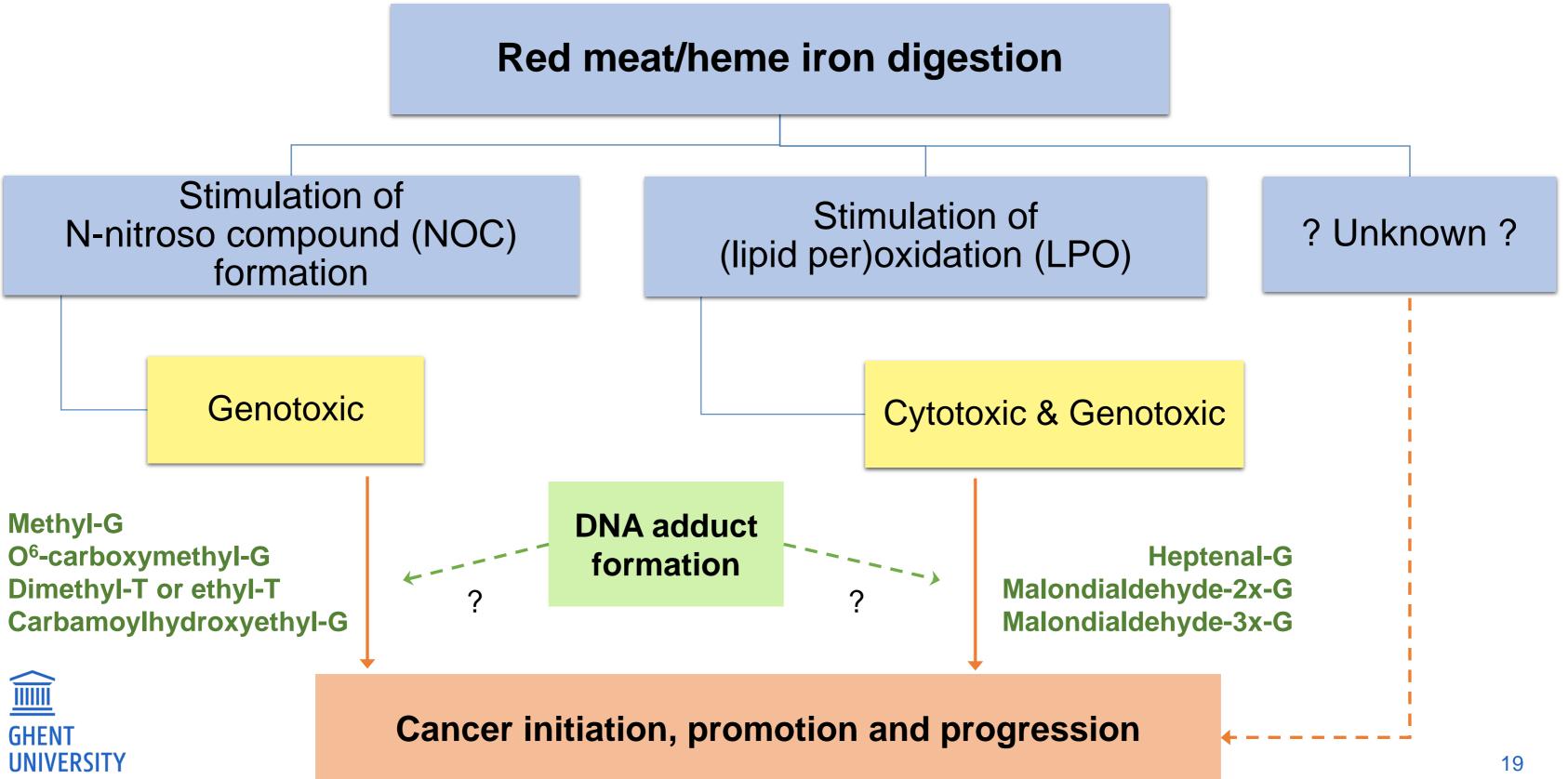
DNA adduct	RT
Trihydroxybutyl-U (-)	0.72
Cro-G (+)	0.96
Carboxyl-A (+)	0.96
Methyl–C (+)	0.96
OHE-C (+)	1.01
1,N2-propano-G (+) of Carboxyethyl-A (+)	1.04
Hydroxy–C (+)	1.10
Nitro-C (+)	1.15
Carboxymethyl-G (-) or Glyoxal-G (-)	1.19
Carboxyethyl-G (+) or Methylglyoxal-G (+) or Carboxyhydroxyethyl-A (+)	1.24
Trihydroxybutyl-T (-)	1.25
Hydroxymethyl–G (+) or Methoxy–G+	1.32
Hydroxybutyl–A (+)	1.63
N2,3-etheno-G (+)	1.71
1,N2-propano-G* (-) or Carboxyethyl-A* (-)	1.75
Carboxyl-A* (+)	1.77
Hydroxymethyl–A (+) or Methoxy–A (+) or Methyl–G (+)	1.85
1,N2-propano-G** (+) of Carboxyethyl-A** (+)	1.93
Methoxymethyl–G (+)	2.21
M2-G (-)	3.56
M2-acetaldehyde-A (-)	3.65
M1-acetaldehyde-A (-)	3.65
Dimethyl-G (+) or Ethyl-G (+) or Hydroxyethyl-A (+) or Methoxymethyl-A (+)	3.66
Hydroxybutyl-A (+)	3.78
Hydroxyhydro-C (-)	3.79
Hydroxybutyl-G (+)	3.83
Dimethyl-G* (+) or Ethyl-G* (+) or Hydroxyethyl-A* (+) or Methoxymethyl-A* (+)	3.88
Hydroxyhydro-C* (-)	4.12
M2-acetaldehyde-A* (-)	4.18
M1-acetaldehyde-A* (-)	4.18
Dodecenoate-C (+)	4.22
Trihydroxybutyl–U (+)	4.27
Hep–G (–)	4.29
Dodecenoate-A (-)	4.35
HydroxyethylC (–) or Methoxymethyl–C (–)	4.37
Dodecenoate-G (-)	4.40
Carbamoylethyl-G (+) of Carbamoylhydroxyethyl-A (+)	4.50
HNE-C (+)	4.61
M3-C (+)	4.64
Hydroxyethyl-C* (-) or Methoxymethyl-C* (-)	4.80
Dodecenoate-A (+)	4.86
Dodecenoate-A* (+)	4.94
Oct-G (-)	5.35
Carbamoylhydroxyethyl-G (+)	5.48
M2-acetaldehyde-G (-)	5.50

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?





PARALLEL RESEARCH: HRMS BASED 'GUT' METABOLOMICS





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

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Polar metabolomics – chemical targets

- Amino acids
- ✤ Amines
- Other N-compounds
- Polyols

*

- ✤ Bile acids
- Carbohydrates
- Short chain fatty acids
- Hydroxy acids
- Multicarboxyl acids
- Monocarboxyl acids





2015

*

Lipidomics – chemical targets

- Fatty acyls * Phospholipids ** Prenols •••• Sterols ** Glycerolipids ** Glycerophospholipids * Polyketides **

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)

Sphingolipids

PARALLEL RESEARCH: METABOLOMICS RED VS. WHITE MEAT



OPEN

27 September 2016 Accepted: 11 January 2017 Published: 14 February 2017 Discovery of 5 discriminating metabolites with potential involvement red meat related diseases

Red meat: carnitine

3-Dehydroxycarnitine

Trimethylamine

Trimethylamine-N-oxide

Modification cholesterol metabolism

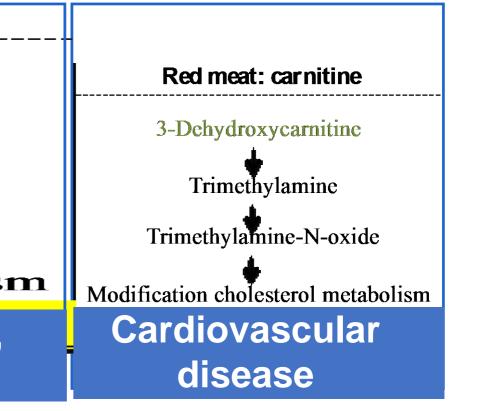
Initiation, promotion and progression of cancer

Progression cancer, diabetes mellitus

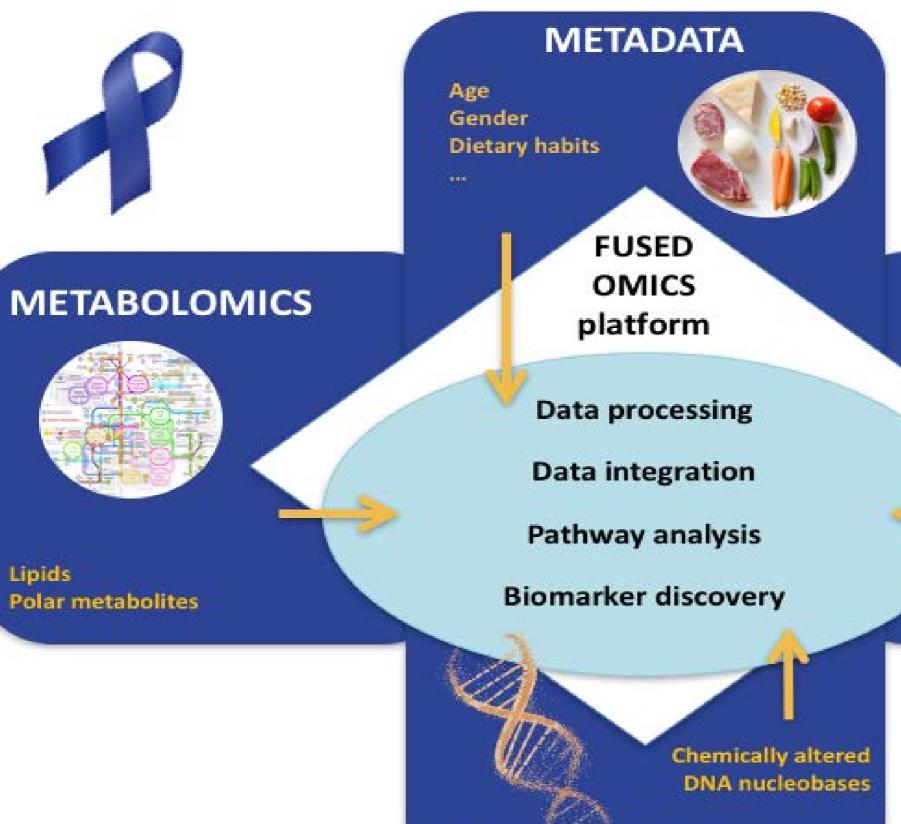


3dehydroxycarnitine

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



FUTURE RESEARCH: FUSED OMICS



DNA ADDUCTOMICS



METAGENOMICS



Composition of the gut microbiome





Lynn Vanhaecke Prof. Dr.

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