MRI
Max Rubner-Institut




Red meat-derived *N*-nitroso compounds, lipid peroxidation compounds and colon cancer

Pablo Steinberg

Max Rubner-institut

The “calm down“ slide

“A well-done steak isn‘t a food choice: it‘s a crime“

The Guardian, 12th Dec 2010
photograph: Branislav Senic/Alamy

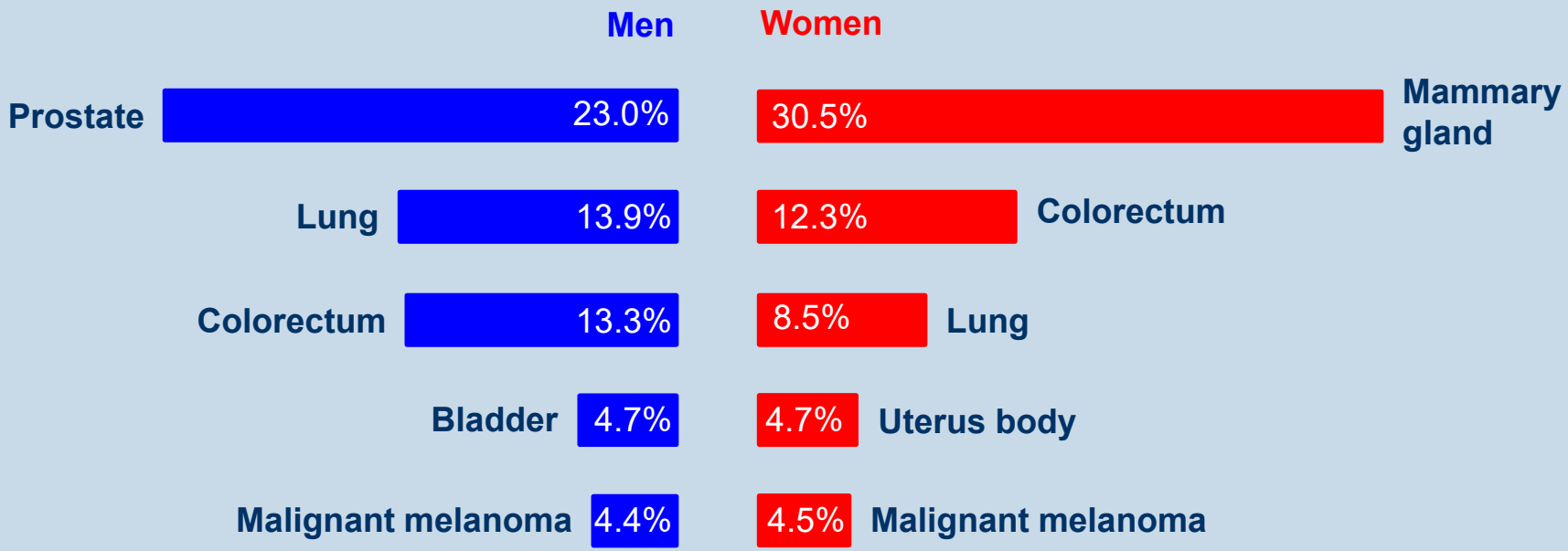
vs.

“My beef isn‘t with beef: why I stopped being a vegetarian“

The Guardian, 19th Jan 2011
photograph: Tim Hayward

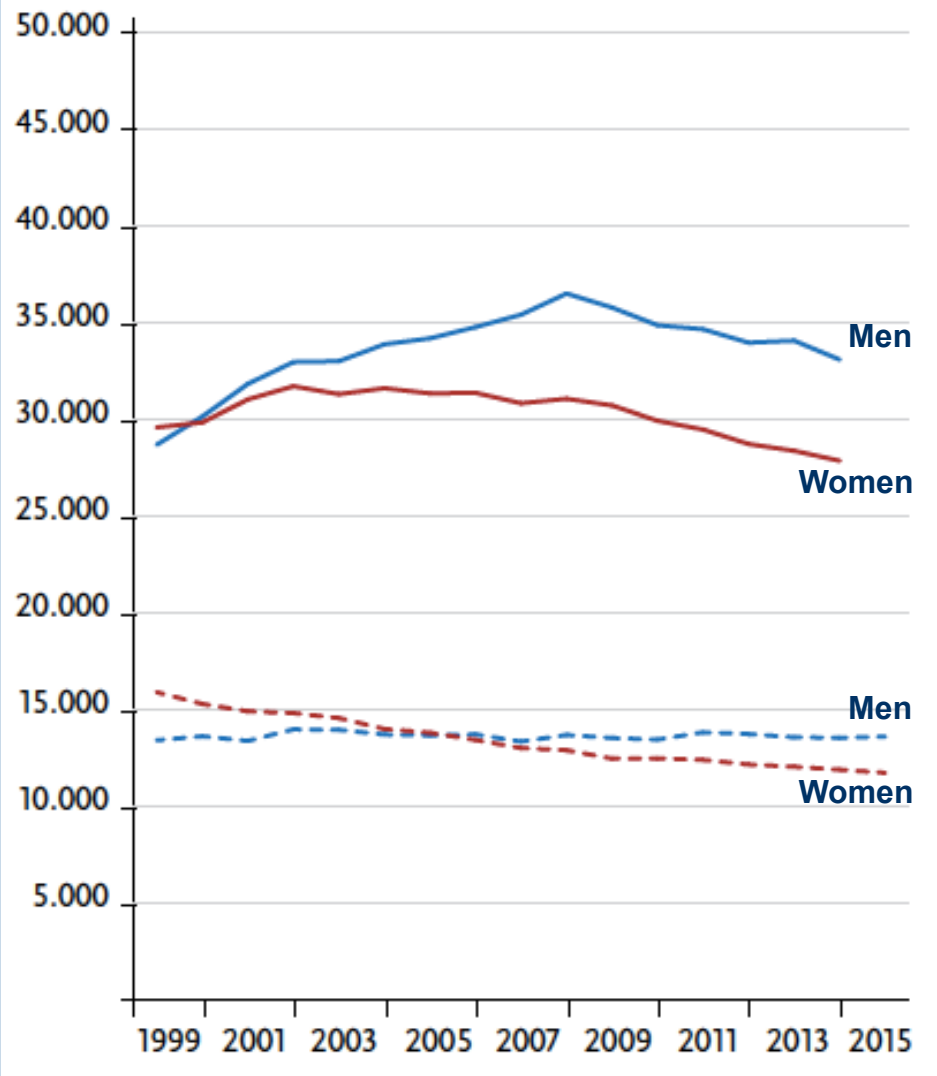
- In 2014, about 480.000 cancer cases were newly diagnosed in Germany.

- Localisation of the five most frequent newly diagnosed cancer cases in Germany in 2014:



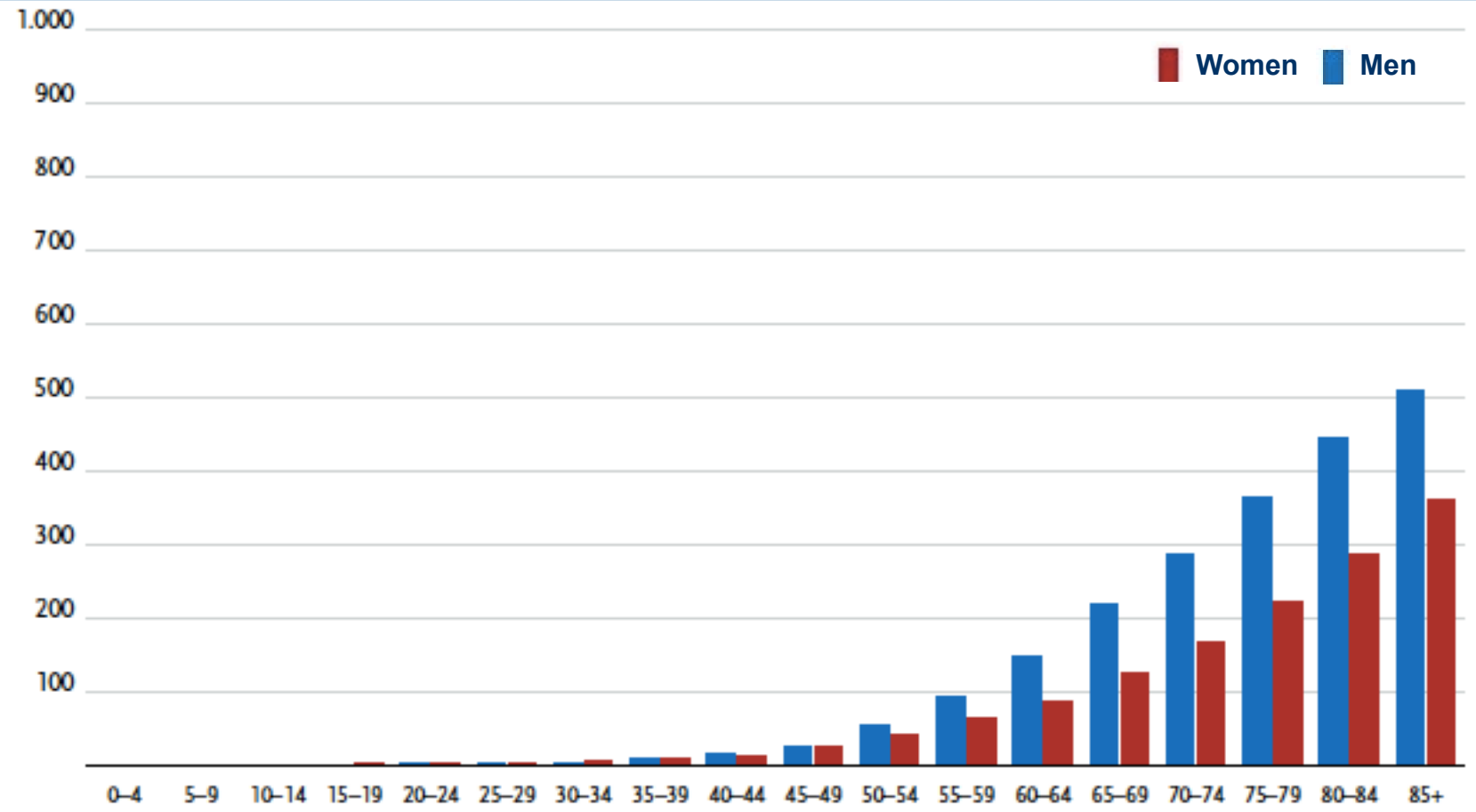
Robert-Koch-Institut (2017)

Number of newly diagnosed CRC cases and number of CRC-associated death cases in Germany (1999-2014/2015)



Robert-Koch-Institut (2017)

Age-specific CRC incidence (per 100,000 inhabitants, in Germany, 2013-2014)

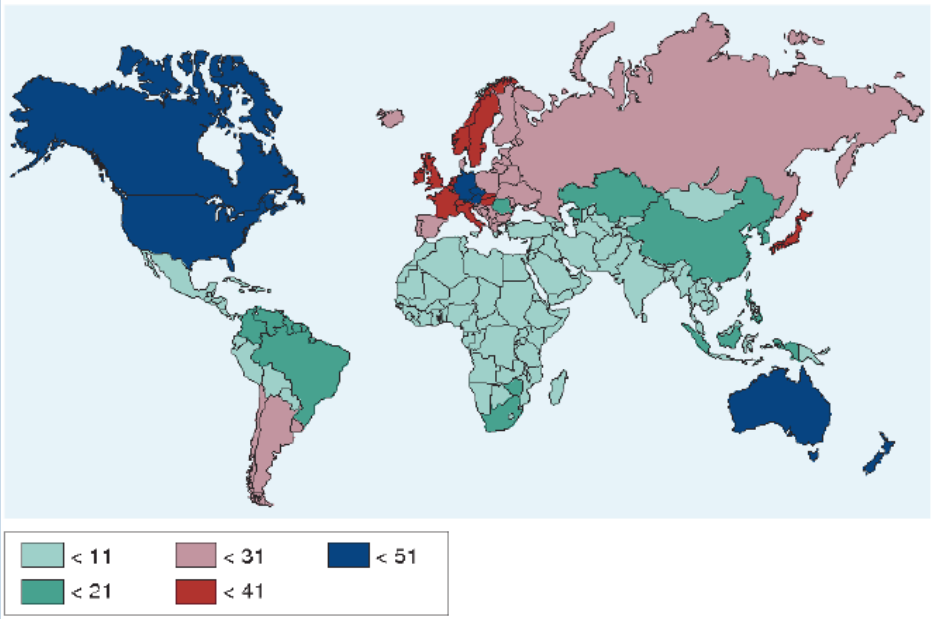


Robert-Koch-Institut (2017)

CRC risk factors

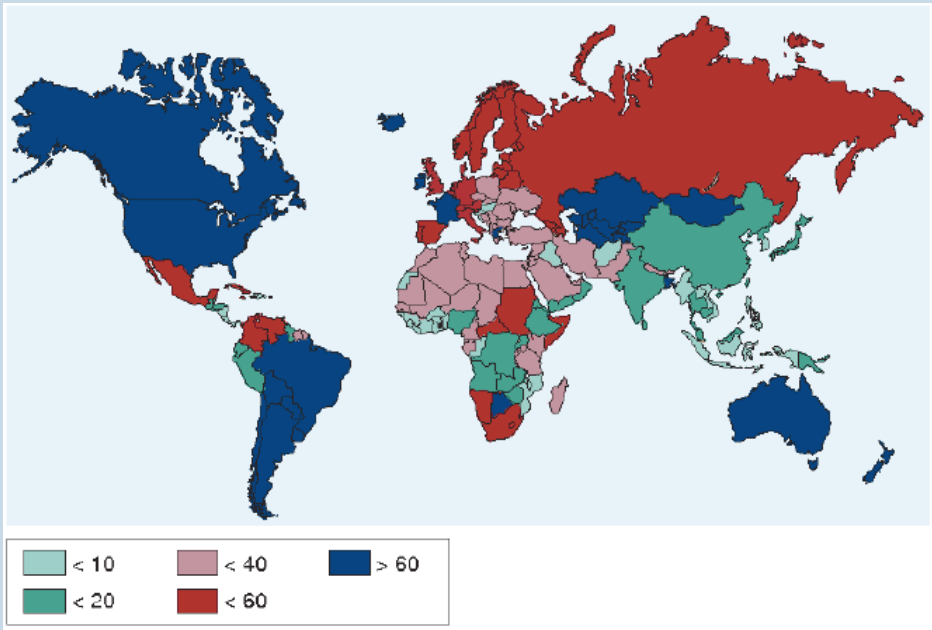
- fiber-poor, fat-rich diet (incl. a high amount of red meat)
- high consumption of alcoholic beverages
- smoking
- genetic predisposition
- chronic inflammatory bowel disease

Diet and Cancer - The European Prospective Investigation into Cancer and Nutrition (Bingham and Riboli 2004)



CRC incidence rates (cases per 100,000 inhabitants)

Estimated red meat consumption (grams/day)



Red meat/processed meat and CRC: Epidemiological evidence

Figure 4.3.3 Red meat and colorectal cancer; cohort studies

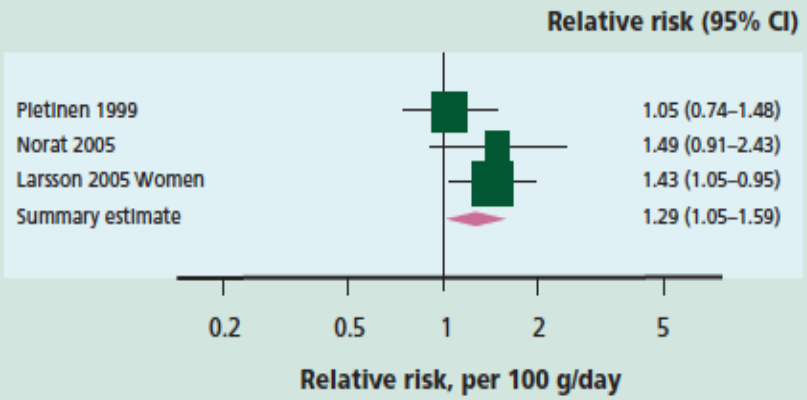
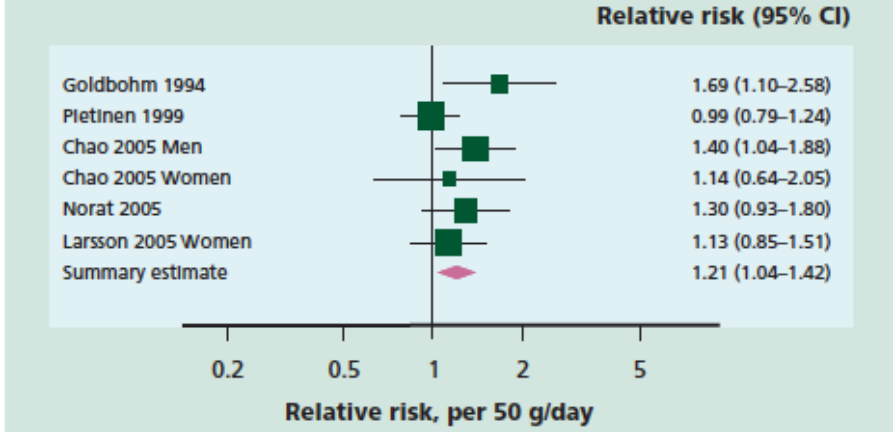
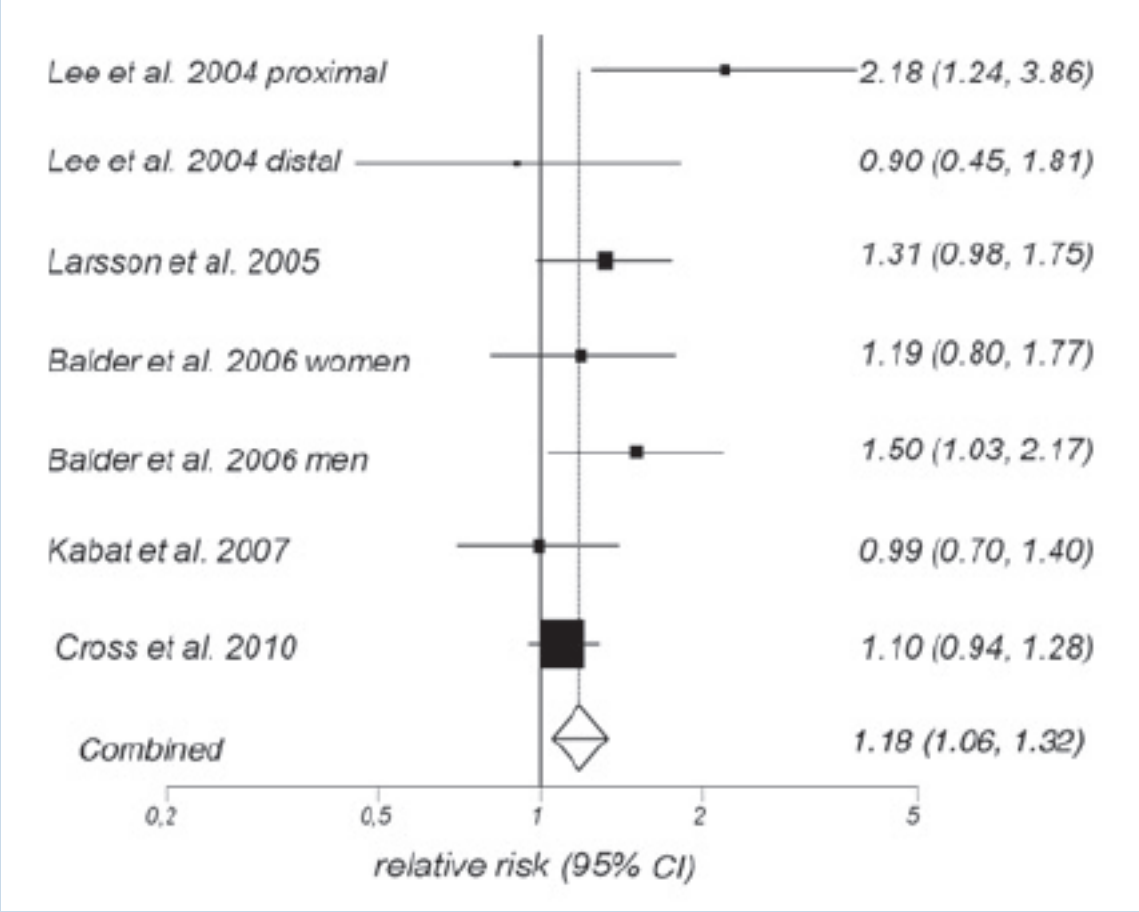


Figure 4.3.6 Processed meat and colorectal cancer; cohort studies



World Cancer Research Fund/American Institute for Cancer Research (2007)

Heme iron and CRC: Epidemiological evidence



Bastide et al. (2011)

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of consumption of red meat. Positive associations have been observed between consumption of red meat and cancers of the colorectum, pancreas, and prostate.

There is *sufficient evidence* in humans for the carcinogenicity of consumption of processed meat. Consumption of processed meat causes cancer of the colorectum. Positive associations have been observed between consumption of processed meat and cancer of the stomach.

6.3 Overall evaluation

Consumption of red meat is *probably carcinogenic to humans (Group 2A).*

Consumption of processed meat is *carcinogenic to humans (Group 1).*

Red meat and CRC: Mutagenic compounds as cause?

- polycyclic aromatic hydrocarbons
- heterocyclic aromatic amines
- exogenously formed *N*-nitroso compounds
- endogenously formed nitroso compounds

Endogenously formed nitroso compounds: First studies (I)

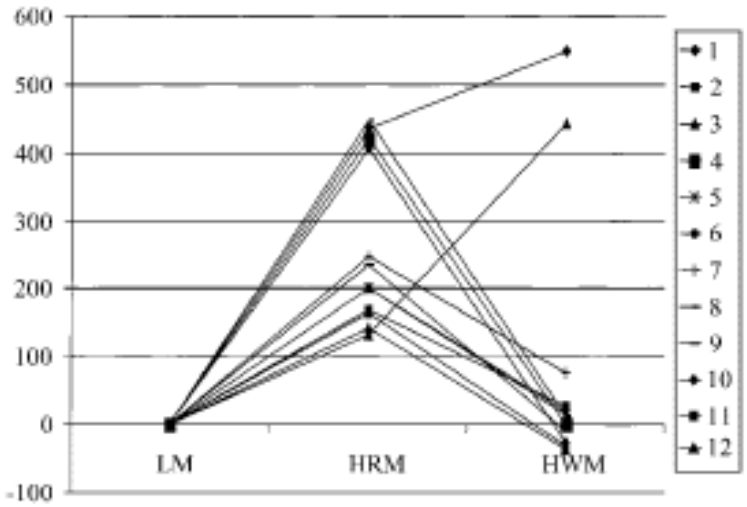


FIGURE 1 Individual changes in fecal ATNC concentration in 12 individuals fed a low-meat (60 g) diet, a high-red meat (420–600 g) diet and a high-white meat (420–600 g) diet. Subjects 1–7 were in group 1 (600 g meat), and subjects 8–12 were in group 2 (420 g meat).

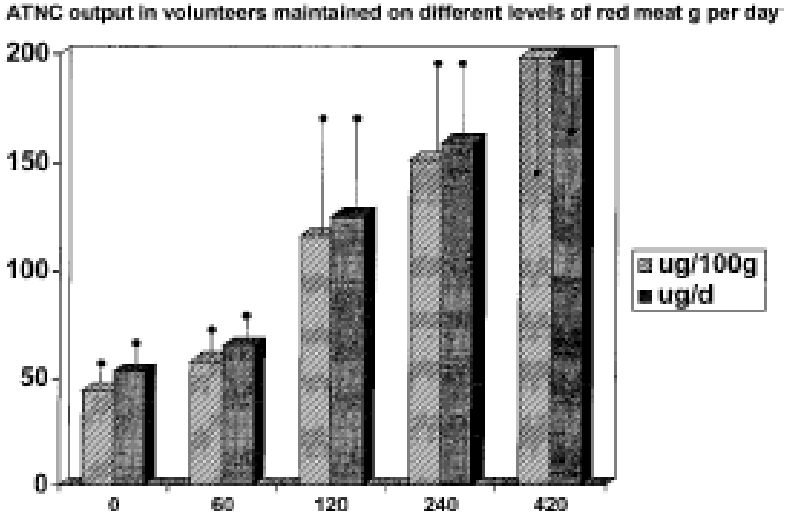


FIGURE 2 Dose response to 0, 60, 240 and 420 g of meat/d and to 120 g of meat/d (from reference 19 and this study). Eight subjects were studied at the 0-, 240- and 420-g level, 9 at the 120-g level and 17 at the 60-g level. Mean and SEM bars are shown.

Bingham et al. (2002)

Endogenously formed nitroso compounds: First studies (II)

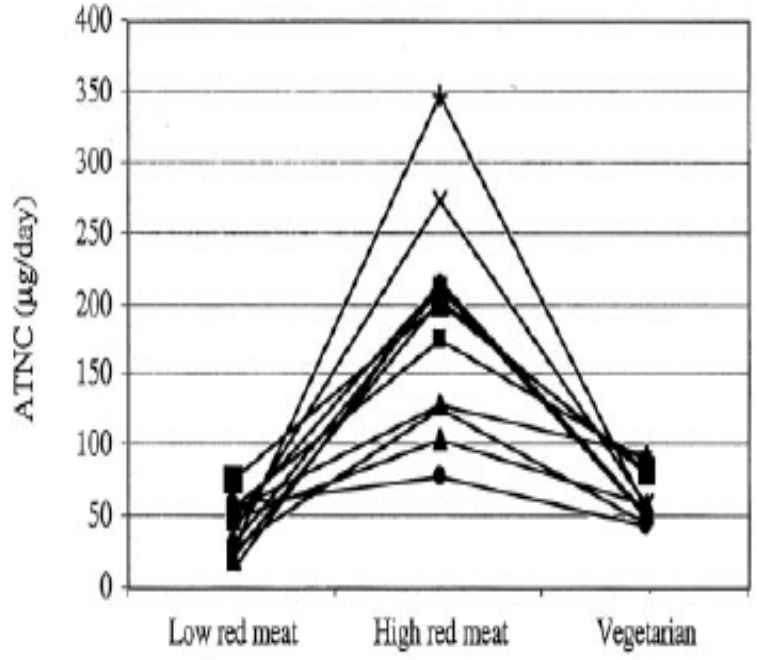


Fig. 1. Protocol 1, mean faecal ATNC excretion for each dietary period (*lines* represent individuals).

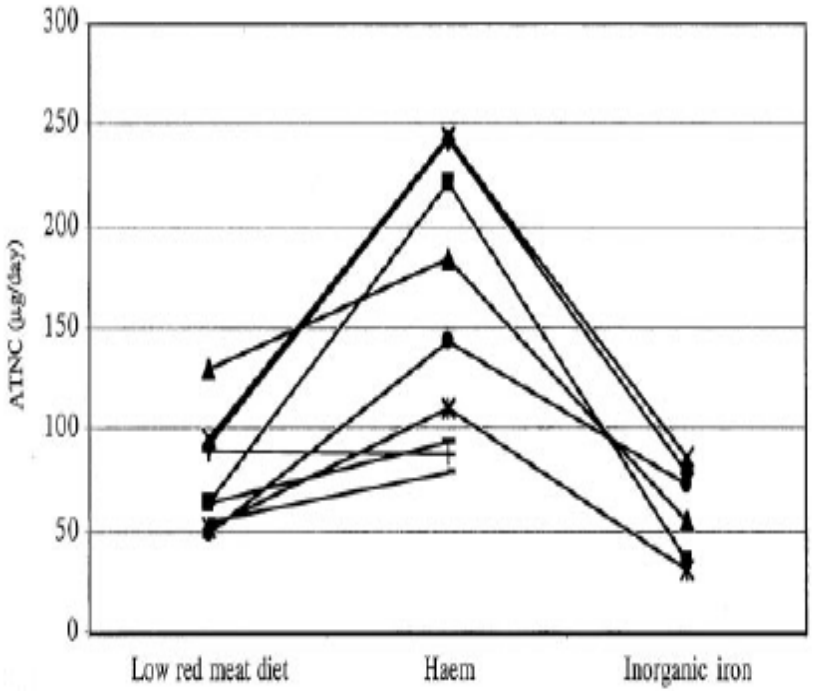
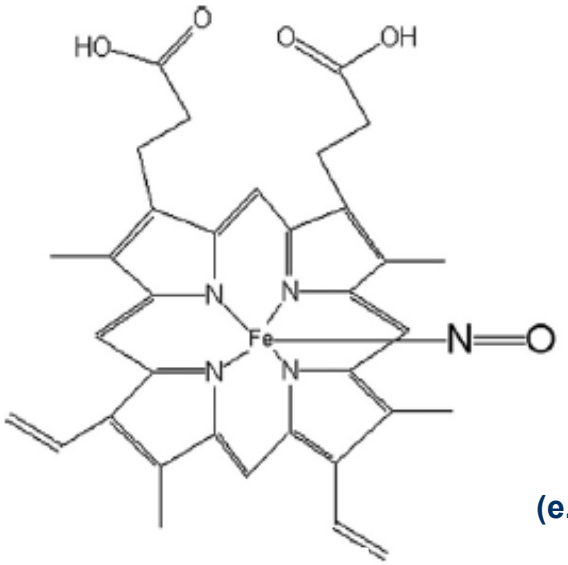


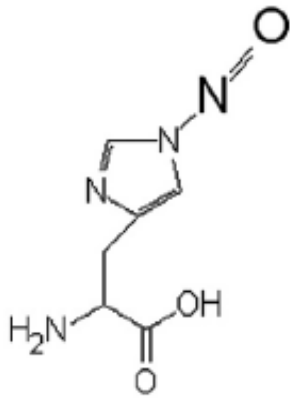
Fig. 2. Protocol 2, mean faecal ATNC excretion for each dietary period (*lines* represent individuals).

Cross et al. (2003)

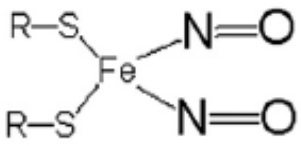
Structurally relevant nitroso compounds



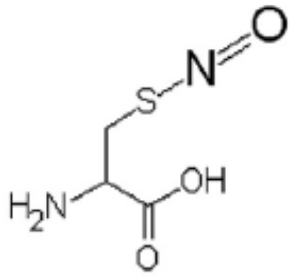
Nitrosyl heme



***N*-nitrosamine**
(e.g. *N*-Nitrosohistidine)

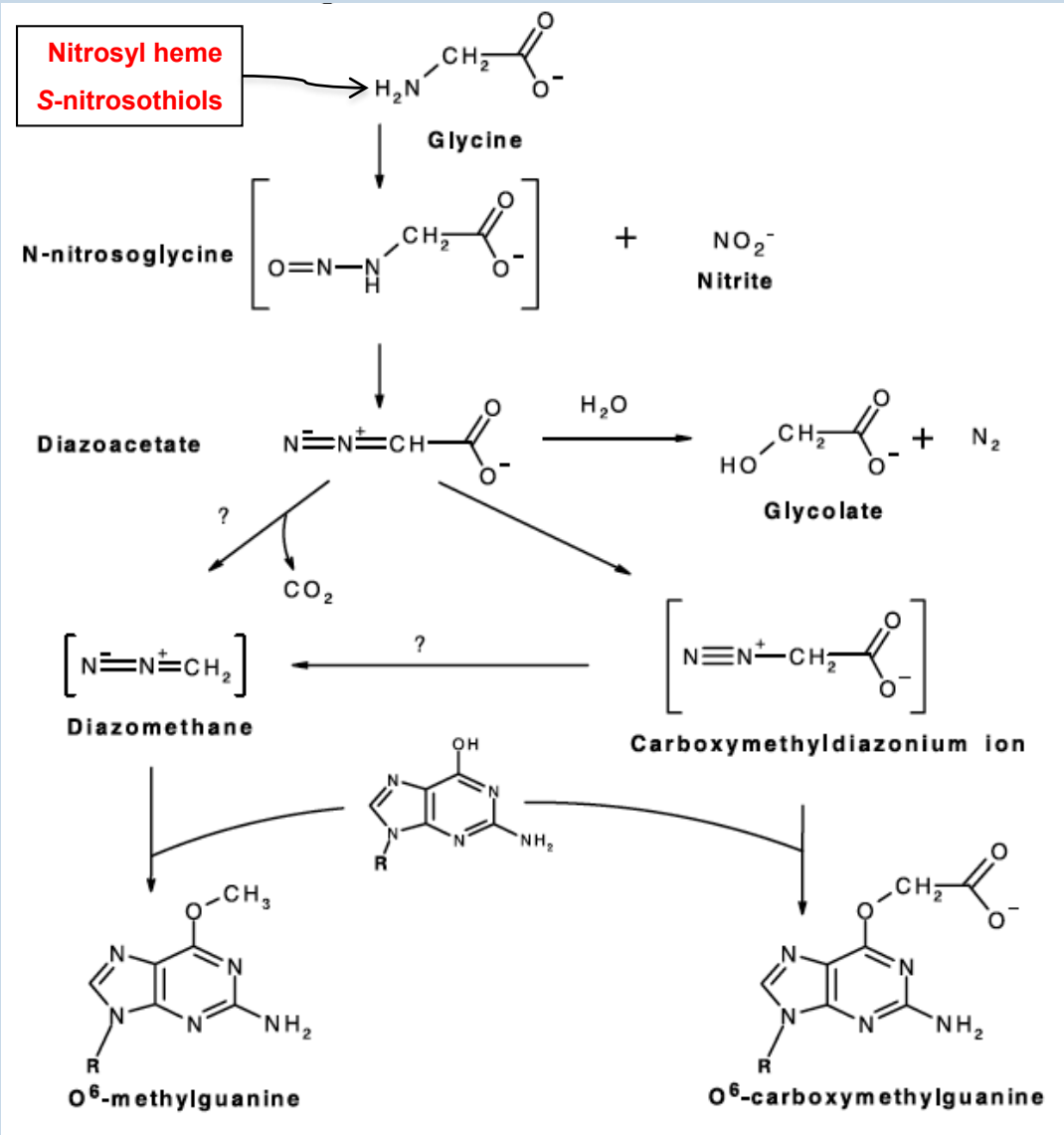


Dinitrosyl iron complex



***S*-nitrosothiol**
(e.g. *S*-nitrosocysteine)

Alkyl guanine adduct formation



Formation of *O*^{6}-carboxymethylguanine adducts *in vivo* (I)

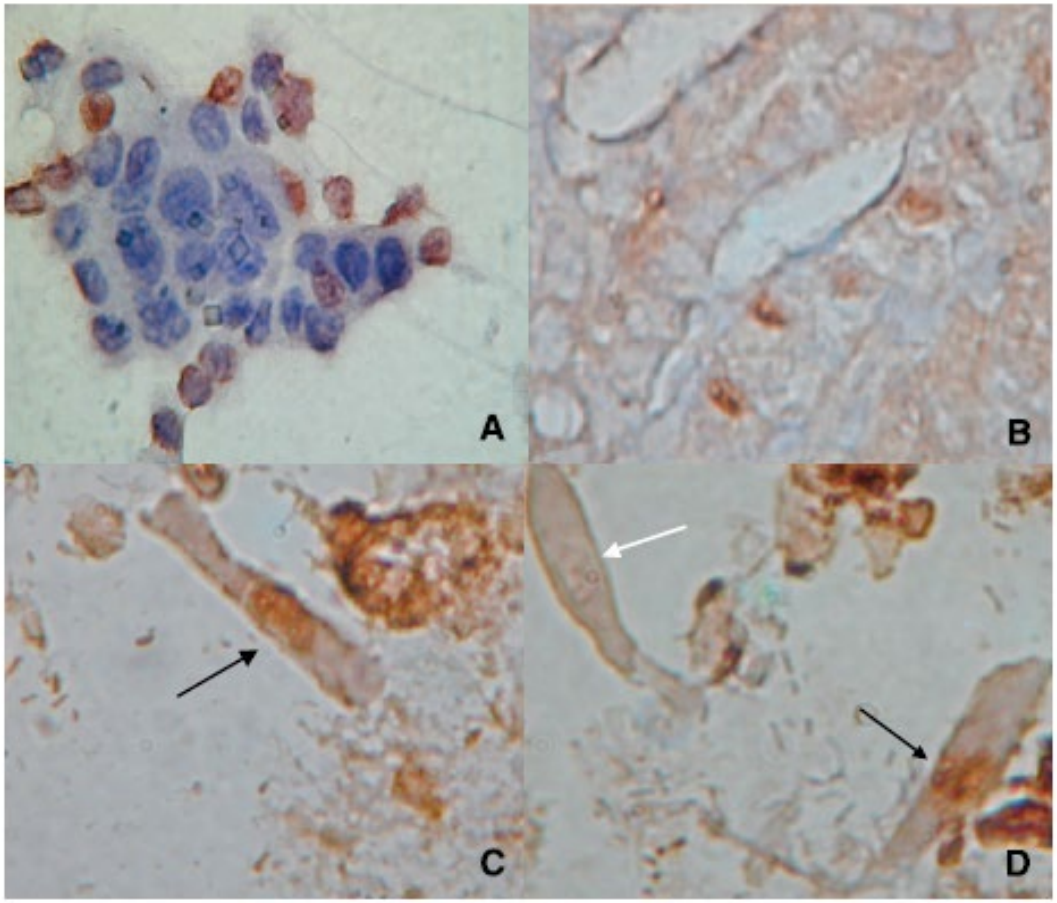


Figure 2. A, positive control for O^{6}CMG identification (HT-29 cultured cells incubated with potassium diazoacetate. Magnification, x400. B, immunohistochemical detection of O^{6}CMG in rat small intestine. Magnification, x400. C-D, presence of exfoliated colonic cells staining positive (black arrows) and negative (white arrows) for the O^{6}CMG adduct isolated from fecal extracts.

Lewin et al. (2006)

Formation of O^6-carboxymethylguanine adducts *in vivo* (II)

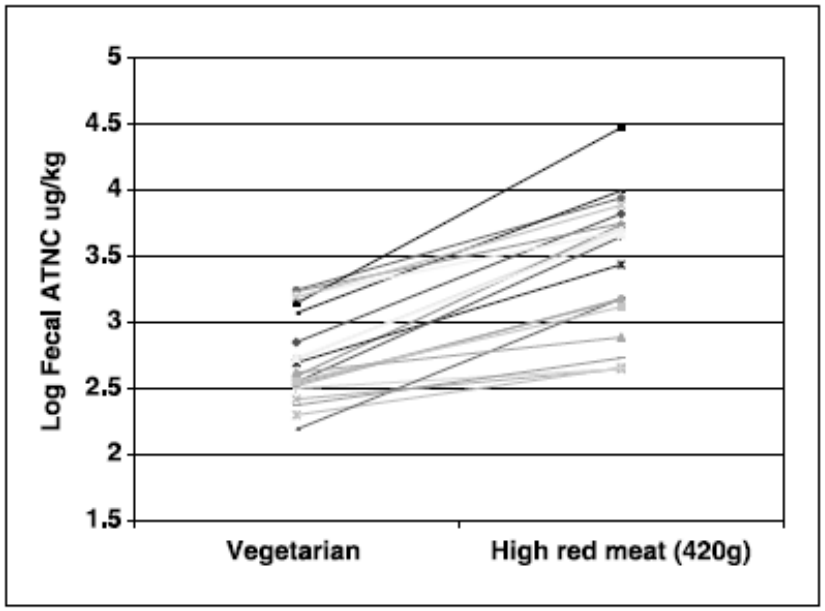


Figure 3. Change in fecal ATNC concentration on changing from a vegetarian to a high red meat (420 g) diet in 21 volunteers.

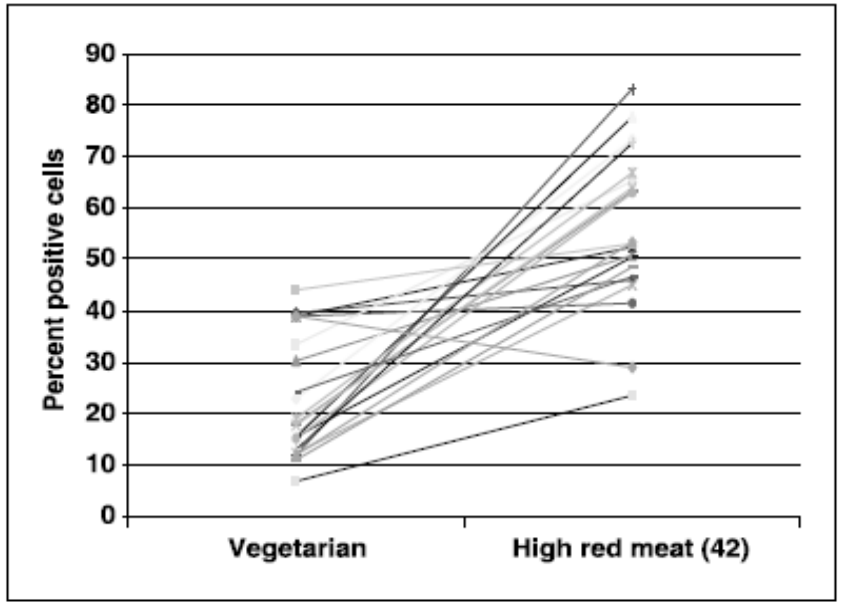


Figure 4. Change in percent cells staining positive for the O^6CMG adduct in exfoliated colonic cells isolated from fecal extracts in 21 volunteers.

Lewin et al. (2006)

Formation of O^6-carboxymethylguanine adducts *in vivo* (III)

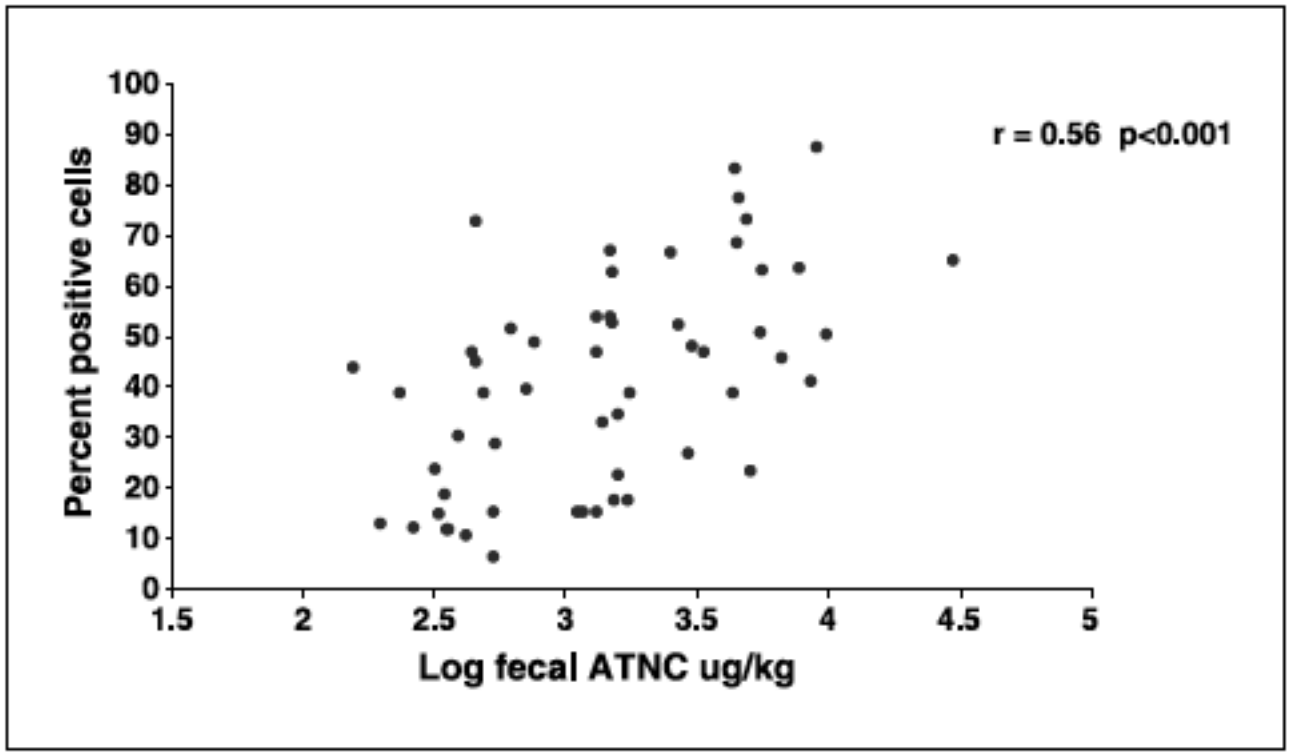
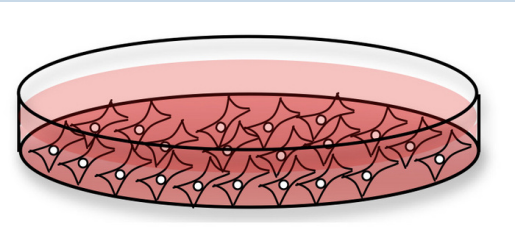


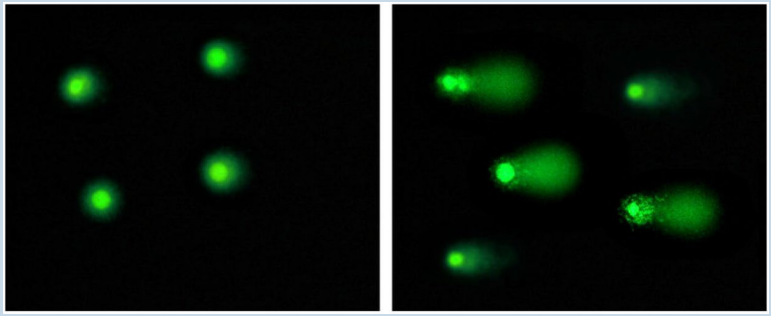
Figure 5. Correlation between fecal ATNC concentration and percent cells staining positive for the O^6CMG adduct in exfoliated colonic cells isolated from fecal extracts (n = 55).

Lewin et al. (2006)

The Comet assay: Principle of the test

Chemical

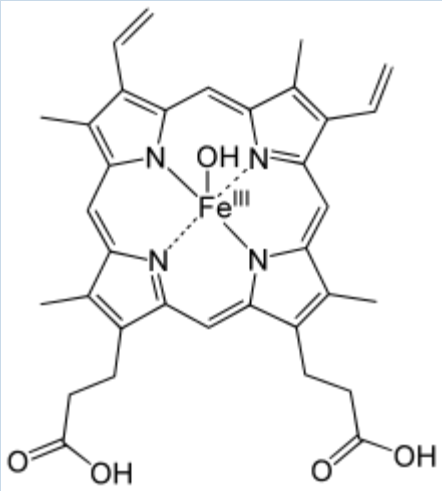




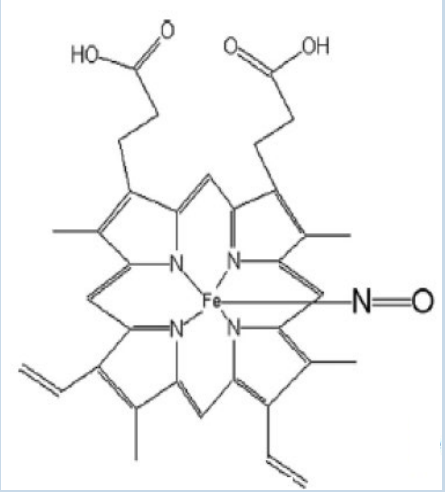
Single cells are embedded on an agarose-coated slide and lysed.

After electrophoresis and fluorescent staining, the damaged DNA is separated from the inatct DNA (the “head“) and generates a comet “tail“.

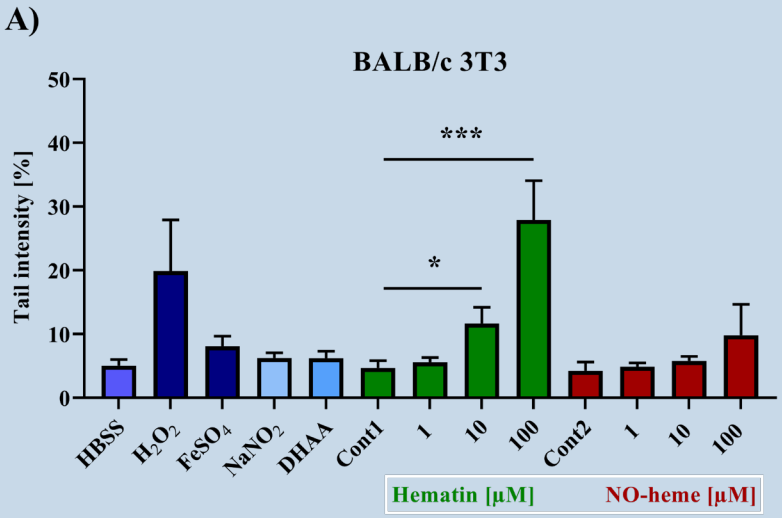
Genotoxicity of hematin and nitrosyl heme: Comet assay

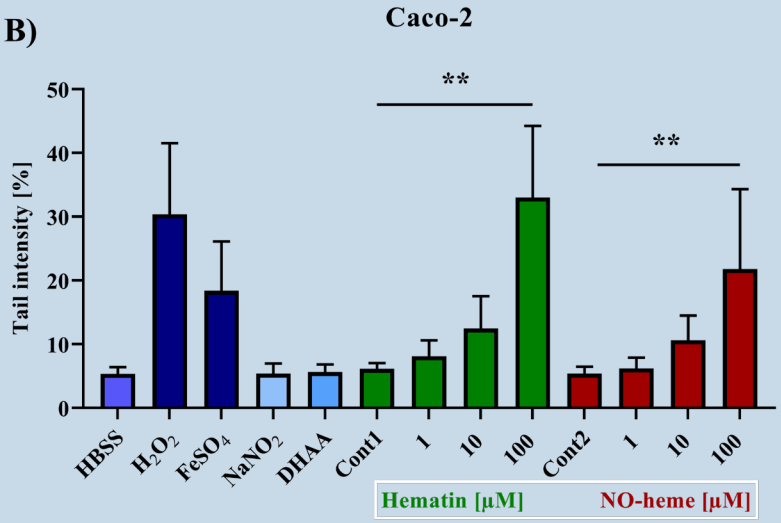


hematin

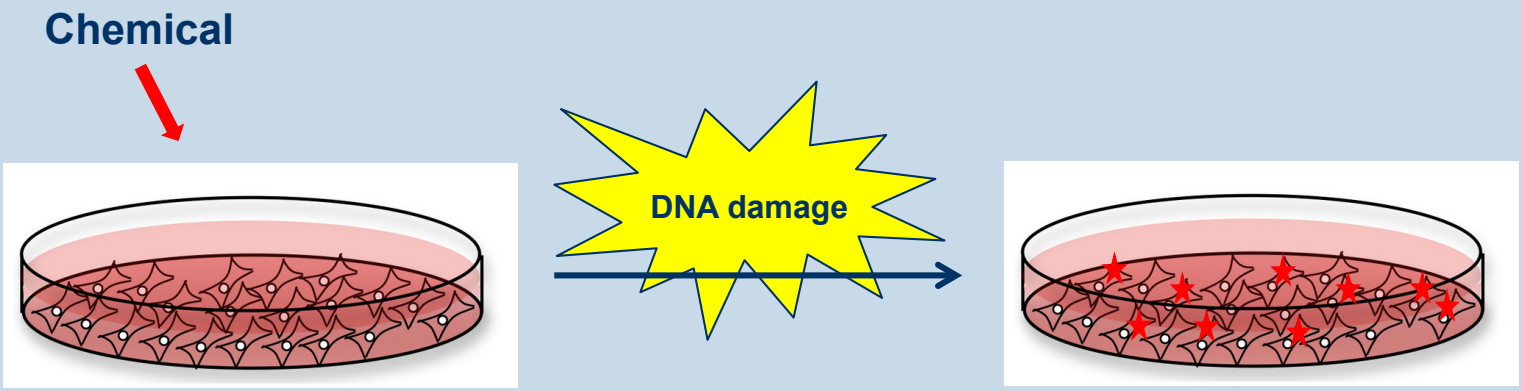


nitrosyl heme



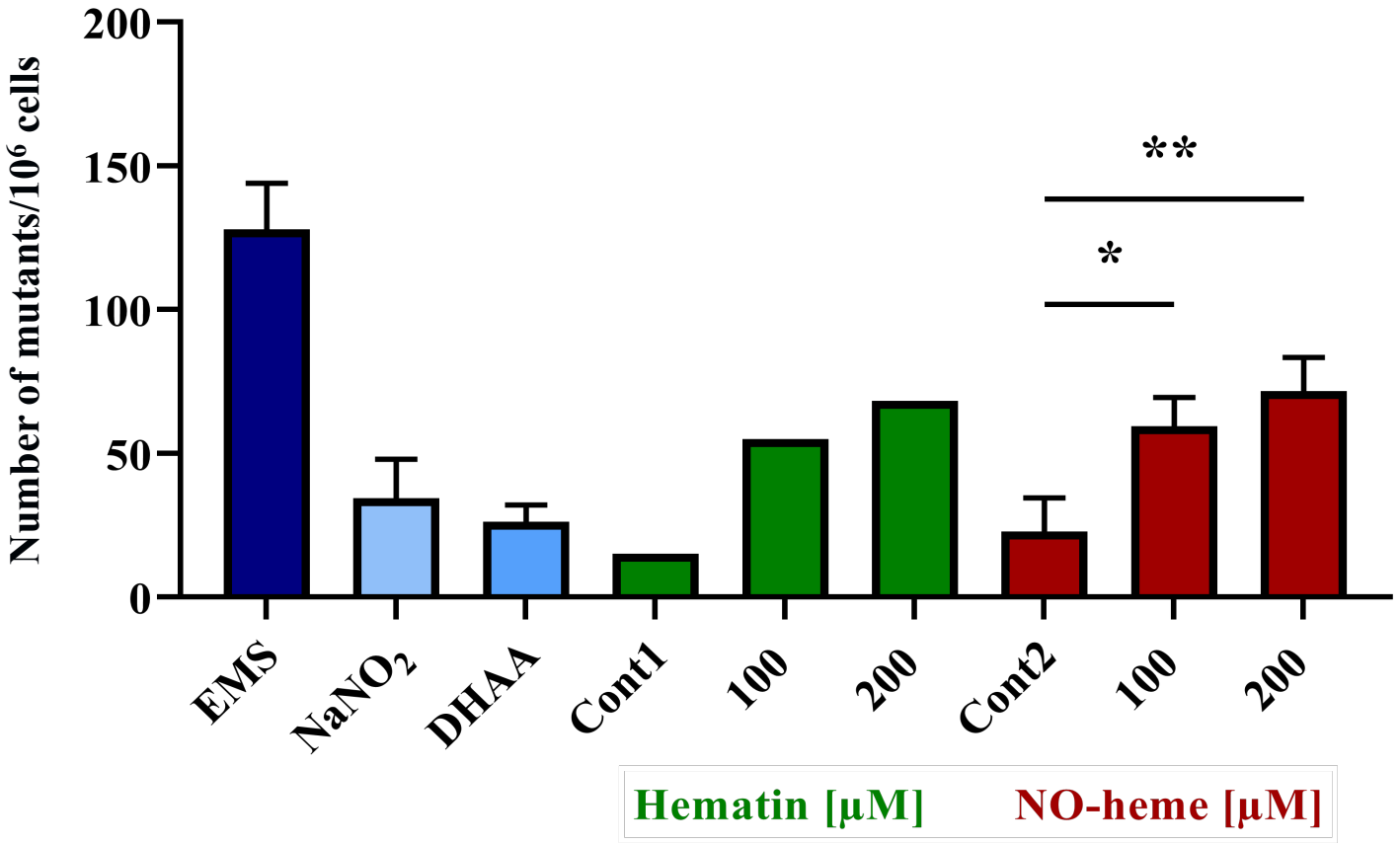


The HPRT assay: Principle of the test

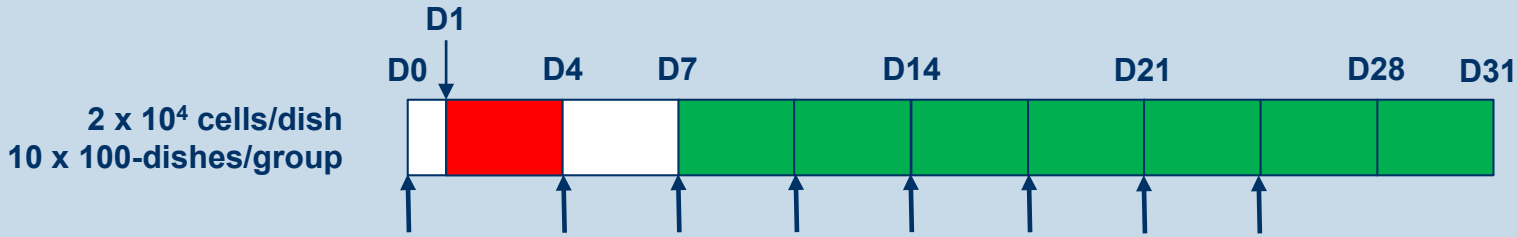


Only the cells with mutated HPRT will survive and are counted.

Mutagenicity of hematin and nitrosyl heme: HPRT assay



The BALB/c 3T3 cell transformation assay: principle of the test

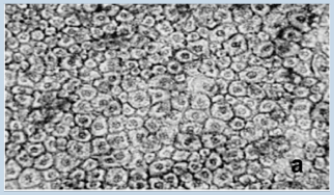


MEM + 10% FBS

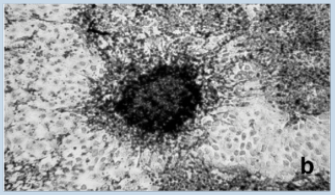
Test chemical treatment

DMEM/F12 + 2 µg/mL insulin + 2% FBS

Medium change



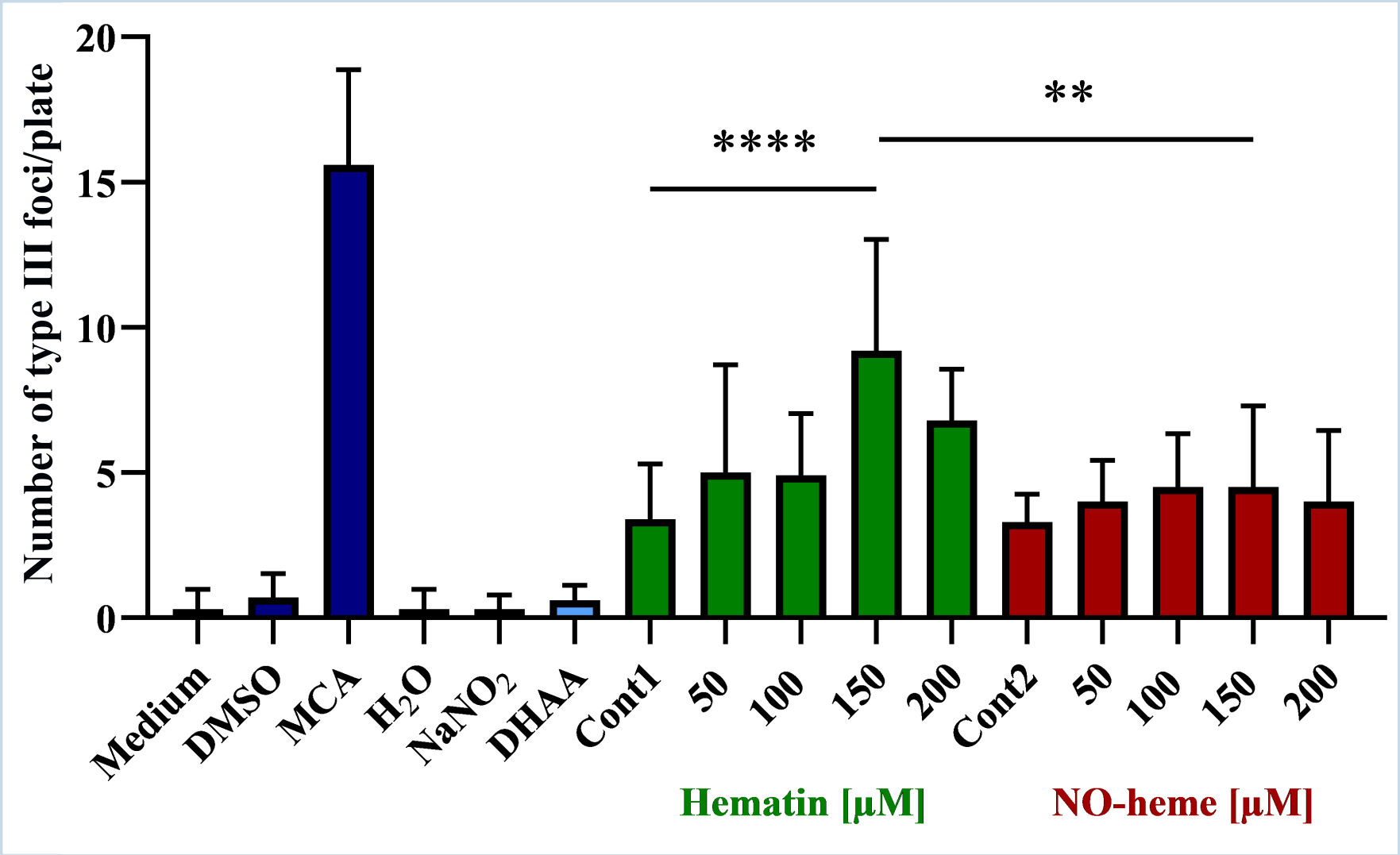
untreated cells



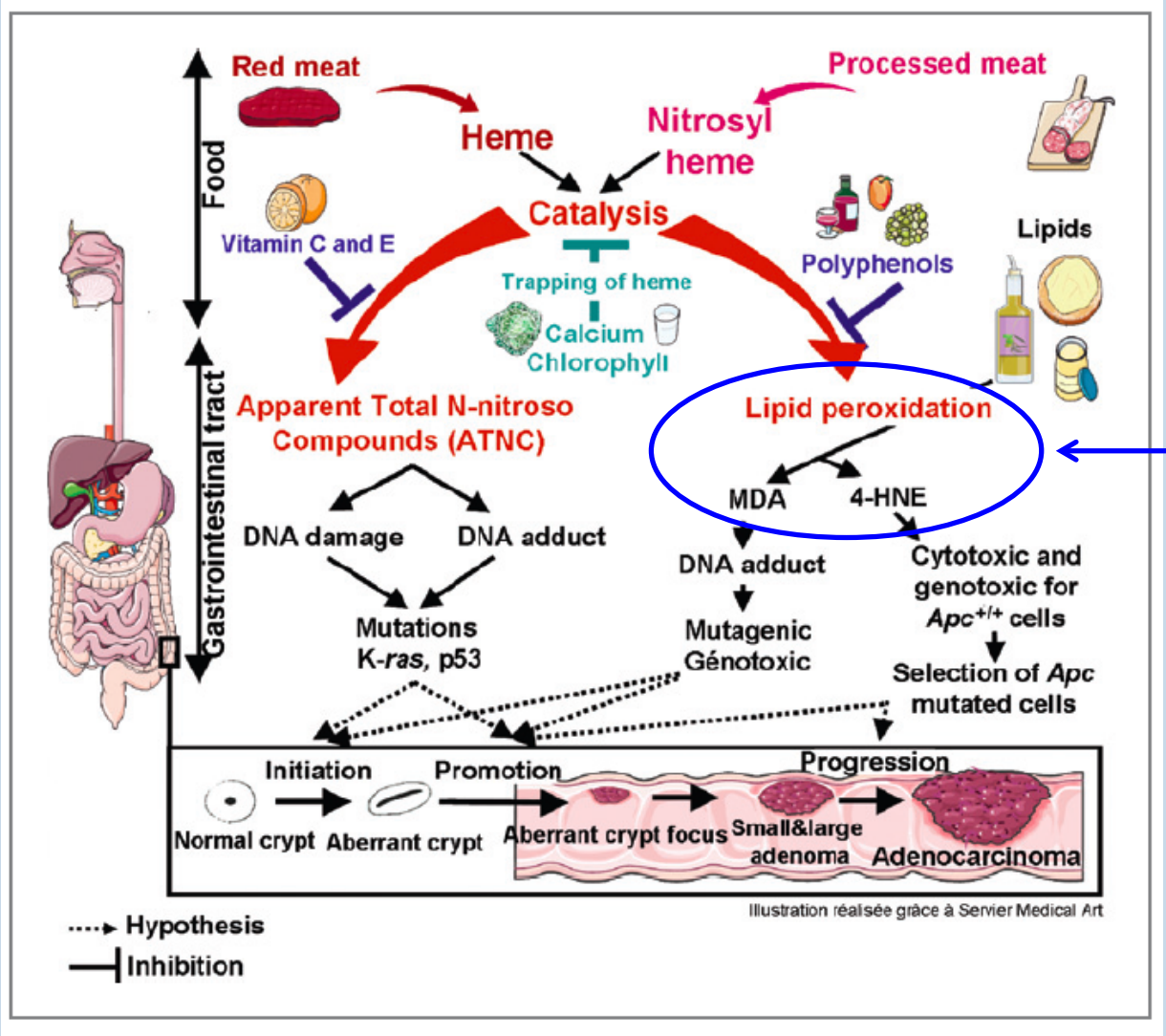
type III focus

Sasaki et al. (2012)

Cell transforming activity of hematin and nitrosyl heme in the BALB/c 3T3 cell transformation assay

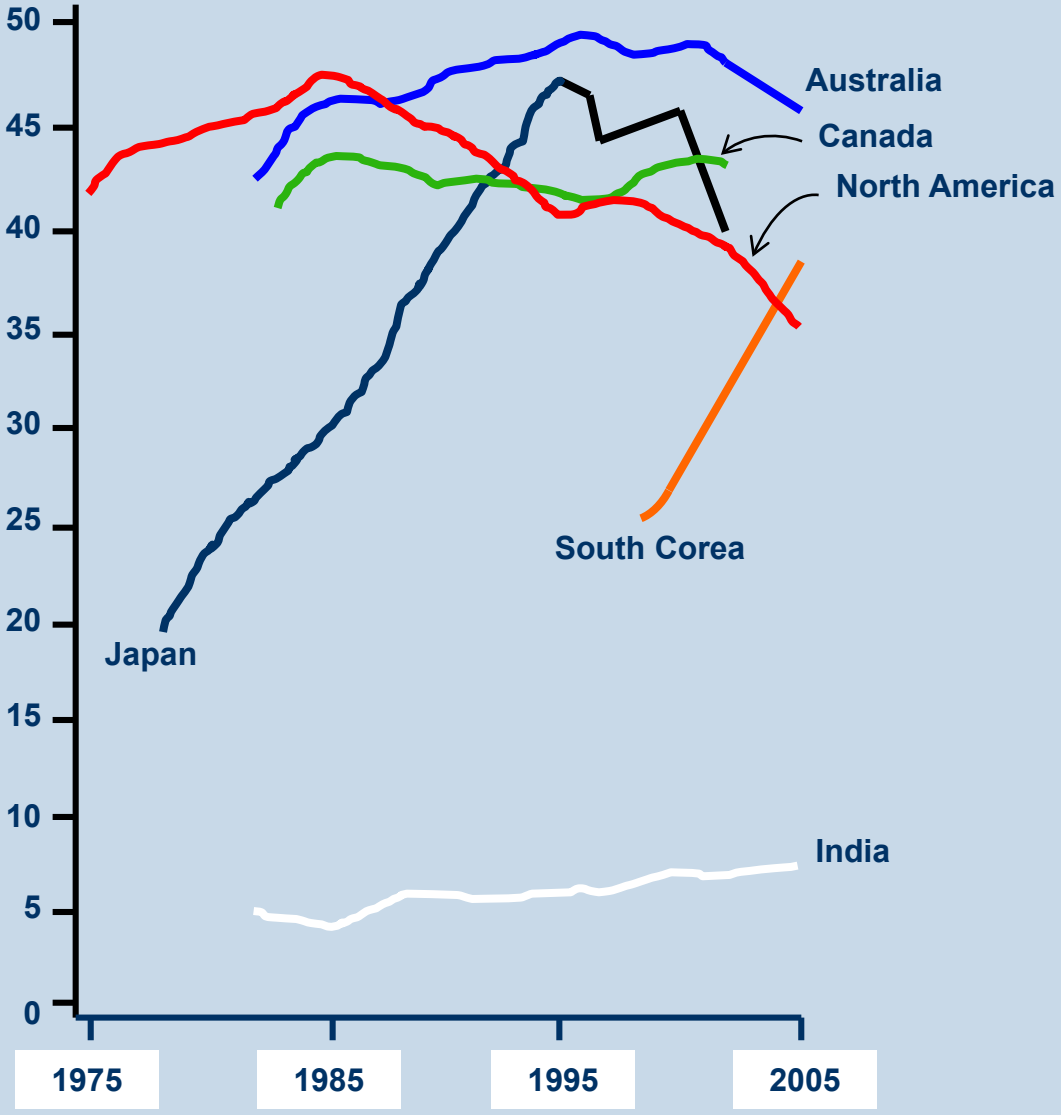


Red meat and colorectal cancer: Further mechanisms of malignant cell transformation



Bastide et al. (2011)

Red meat and colorectal cancer: Further mechanisms of malignant cell transformation - viruses?

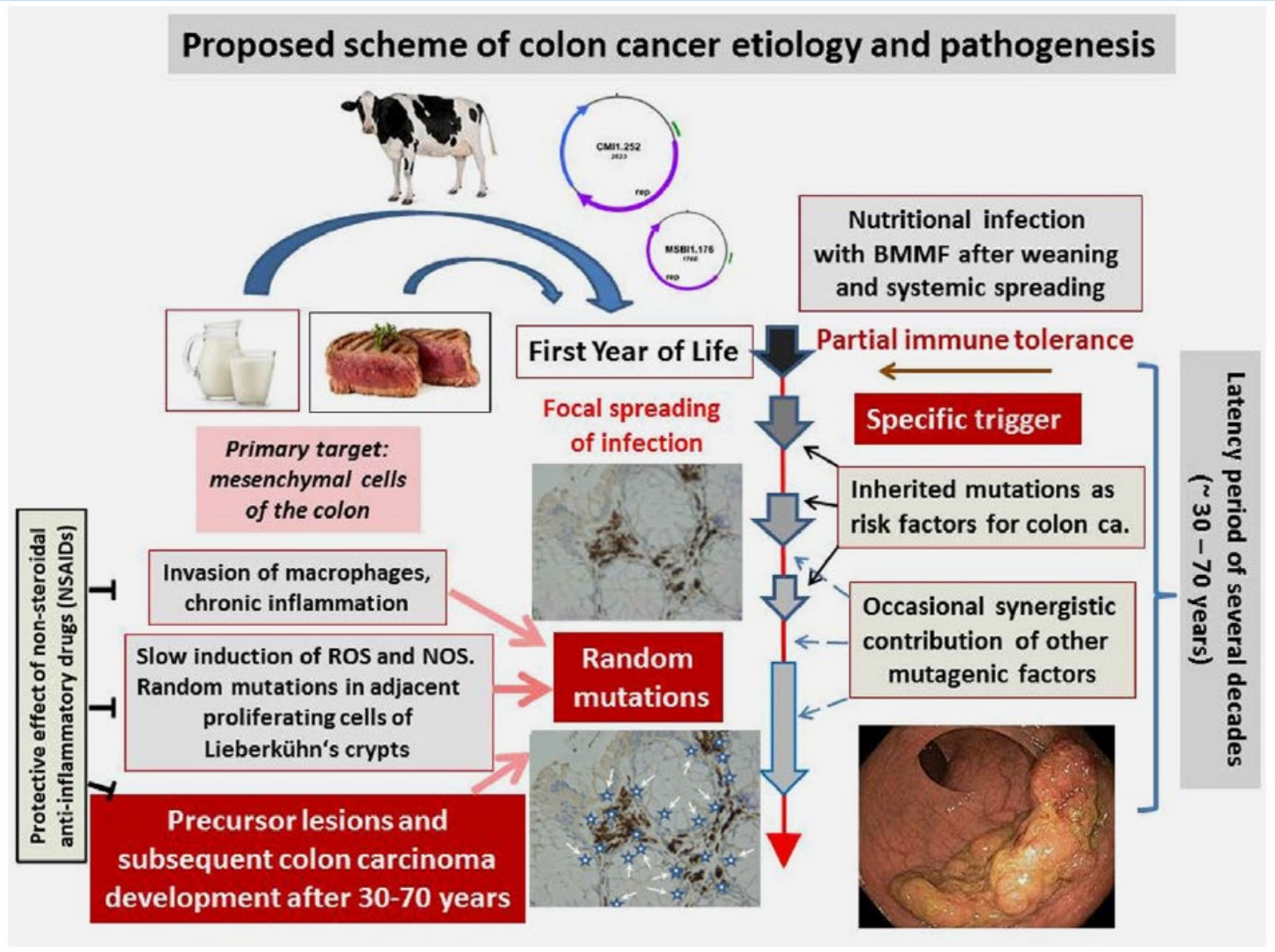


Shabu-shabu (Japan)

Yukhoe (South Korea)

zur Hausen et al. (2012)

“Infectious plasmidom“: A new etiologic agent for CRC?



zur Hausen et al. (2019)

Conclusions (I)

- **The consumption of red and processed meat, but not that of white meat and fish, are associated with an increased CRC risk.**
- **Meat ist particularly rich in heme and heme leads to the formation of endogenous nitroso compounds.**
- **Nitrosyl heme and *S*-nitrosothiols quantitatively are the main endogenously formed nitroso compounds, and their formation strongly increases after consumption of red meat.**
- **Nitrosyl heme and hematin lead to DNA damage (as shown in the Comet assay) and induce mutations (as shown in the HPRT assay) in mammalian cell lines.**
- **Hematin, but not nitrosyl heme, is able to malignantly transform BALB/c 3T3 cells.**

Conclusions (II)

- **The results obtained suggest that iron-mediated lipid peroxidation coupled to the subsequent formation of reactive oxygen species are the driving force in the heme-induced malignant cell transformation process.**
- **The recommendation of organizations such as the German Nutrition Society is to limit the consumption of red meat (including cold cuts) to maximally 600 grams per week in order to reduce the CRC risk in humans.**
- **The parameters to be taken into account when considering the health risk of individuals consuming red meat are the meat "dose" and quality and, by far, the individual *dietary pattern*.**

MRI
Max Rubner-Institut




Thank you very much for your attention!