

Effect of dietary meat and fish on endogenous nitrosation and colorectal cancer

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1 Introduction

Colorectal cancer (CRC) is one of the most common types of cancer with more than 30,000 new cases, and 14,000 deaths, per year in Germany¹. Diet has been identified as an important contributing factor, in particular red and processed meat (RR 1.22 (95% CI 1.11 – 1.34) per 100 g/d)^[1]. Several hypotheses have been proposed to explain this association, for example the formation of heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons (PAH), the pro-carcinogenic activity of haem^[2] or the endogenous formation of nitroso compounds (NOC)^[3]. The exposure to HCA and PAH does not explain the differences observed between red and white meat, whereas haem content varies considerably^[4], and it is therefore a more likely contributing factor. It has cytotoxic and haemotoxic effects, and can increase colonic epithelial proliferation in rats^[5]. However, most of these effects are only observed at haem concentrations equivalent to a daily consumption of more than 600 g red meat. Haem-induced lipid-peroxidation or haem-promoted formation of nitroso compounds are the most-likely mechanisms explaining the association between red meat and colorectal cancer^[2]. Lipid-peroxidation results in the formation of malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE)^[6], which can promote cancer^[7]. Endogenous nitrosation reactions result in the formation of carcinogenic *N*-nitroso compounds and the subsequent formation of DNA adducts. Previous *in vitro* studies have shown that mutations induced by compounds linked to endogenous nitrosation are similar to those found in colorectal tumours^[8], supporting the hypothesis that endogenous nitrosation, rather than lipid peroxidation, is the mechanism underlying the link between meat and cancer.

2 Endogenous formation of nitroso-compounds

Red and processed meat consumption results in an intake-dependent endogenous formation of nitroso compounds, which are commonly referred to as *apparent total N-nitroso compounds* (ATNC)^[9]. Although details of the chemical nature of ATNC was initially unknown, it was assumed that due to their similarity with *N*-nitroso compounds they have carcinogenic properties. Using refined analytical methods^[10], it was possible to show that the main contributors to ATNC following a high meat diet were nitrosyl-haeme and nitroso thiols, while only small amounts of other nitroso compounds were detected (Figure 1).

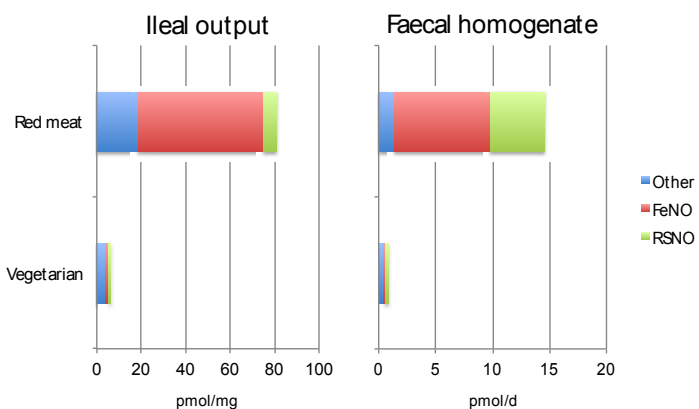


Figure 1 Different types of faecal nitroso compounds following a vegetarian or a high meat in ileal output and faecal homogenates following either a high meat or a vegetarian control diet. Data from Kuhnle *et al.* (2007)

¹ WHO/IARC data (GLOBOCAN) for 2008

2.1 Detection of endogenously formed nitroso compounds

Most methods used for the detection of endogenously formed NOC use chemiluminescence to detect chemically released NO. This method, without any pre-treatment of the sample except for stabilisation and removal of nitrite yields the total amount of NOC present, referred to as ATNC. Nitroso thiols and nitrosyl haem can be detected by using selective chemical denitrosation with mercury(II)chloride (for nitroso thiols) and potassium ferricyanide (for nitrosyl haem). Although this method is generally very reliable, some nitroso compounds – in particular nitroso-thiols – are very sensitive to the presence of metal ions or exposure to UV light, and careful sample handling is important to obtain reliable results.

2.2 Underlying mechanism

The mechanisms underlying the meat-induced endogenous formation of nitroso compounds have not yet been completely elucidated. Data from dietary intervention studies suggested that dietary haem can promote the formation of nitroso compounds ^[11], however, *in vitro* ^[10] studies showed that a combination of haem and free thiol groups are necessary for endogenous nitrosation reactions to occur. Indeed, while the removal of the haem group from haemoglobin resulted only in a reduction of NOC formation by 24%, blocking free thiol groups reduced their formation by more than 80%; the addition of free haem as haemin increased the formation of nitroso-thiols by 50%.

The rapid formation of nitroso thiols under acidic conditions can be explained by the large rate constant ($k=465,000 \text{ M}^{-2}\text{s}^{-1}$) for acid catalysed nitrosation, in particular when compared to the constant for N-nitrosation

($k=4600 \text{ M}^{-2}\text{s}^{-1}$) ^[12]. The nitrosylation reaction of haem is more complicated and it is therefore difficult to compare rates ^[13]. However, the reductive and anaerobic environment in the small intestine helps to maintain the haem iron in its ferrous state and facilitates its nitrosylation by nitrite or NO ^[14]. Both nitrosyl haem and nitrosothiols are known to act as NO donors ^[15] and both can act as nitrosating agent ^[16]. It is therefore likely that these compounds promote the formation of N-nitroso compounds or other highly reactive alkylating agents, which can result in the formation of DNA adducts. A summary of this putative pathway is shown in Figure 2. Furthermore, these compounds could promote a state of low-grade inflammation, as risk factor of cancer ^[17], either directly ^[18] or by causing oxidative and nitrative damage ^[19].

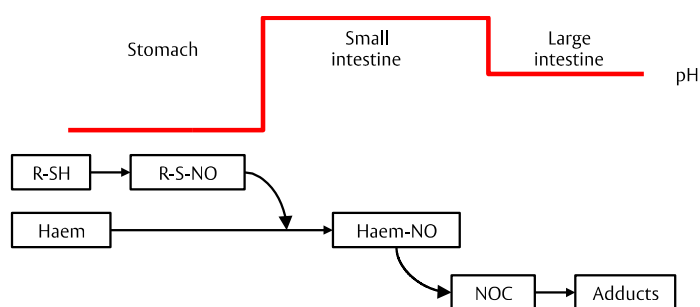


Figure 2: Putative mechanism of meat-induced endogenous formation of nitroso compounds and subsequent formation of DNA adducts.

2.3 Red meat, fish and inflammation

Fish intake has been associated with a reduced risk of colorectal cancer ^[20], although the underlying mechanism is not known. ω -3 fatty acids might have an effect, e.g. by reducing inflammatory processes, but data from observational studies are inconclusive ^[21]. In a dietary intervention study, no statistically significant difference in inflammation markers between meat and oily fish diets have been observed ^[22]. This study also showed that oily fish did not affect the formation of NOC, and the reduction observed can be explained by the reduction in dietary meat.

2.4 Endogenous nitrosation, DNA adducts and mutations

Endogenously formed nitroso compounds can react with DNA bases which results in the formation of DNA adducts and can lead to mutations. Diazoacetate (nitrosated glycine), which cannot be detected using the methods described above, has been shown to react with guanine to form two different adducts, O⁶-methylguanine (O⁶-mG) and O⁶-carboxymethylguanine (O⁶-CmG) [23]. In human dietary intervention studies, a strong correlation between endogenous NOC formation and O⁶-CmG adducts has been found in exfoliated colonocytes [24] and *in vitro* studies have shown that the pattern of mutation induced by diazoacetate is similar to those found in gastro-intestinal tumours [8]. Furthermore, a recent study in 185 archived CRC tumour samples from EPIC Norfolk showed a strong association between meat intake and GC-to-AT mutation (OR 1.68, 95% CI 1.03–2.75 per 19g/day) [25]. This data supports the hypothesis that endogenous nitrosation reactions, leading to the formation of alkylating agents and DNA adducts, are a likely explanation for the association between red and processed meat and cancer.

3 Summary and future work

Dietary intervention studies, as well as animal and *in vitro* studies, have confirmed that red and processed meat intake results in the endogenous formation of NOC and that these can lead to the formation of DNA adducts and mutations. However, there is still a paucity of data to confirm that endogenous NOC formation is the mechanism underlying the association between red and processed meat and cancer. To establish this link, it is important to address the following:

- The underlying mechanisms are still not completely understood. While it is known that free thiol and haem groups are necessary for NOCs to form, the mechanism of their formation *in vivo*, and subsequent DNA adduct formation, remains unknown. Understanding these mechanisms is not only important to establish the link between red and processed meat and CRC, but also to investigate methods to control NOC formation.
- It is currently not possible to investigate NOC formation in observational studies. A previous study investigating the association between endogenous nitrosation and CRC risk relied on estimate exposure [26], using the iron content from meat to predict endogenous nitrosation. The development of a reliable serum or urinary biomarker of endogenous nitrosation would allow to investigate associations between cancer risk and endogenous nitrosation in epidemiological studies.

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