

Heme Iron from Meat and Risk of Colorectal Cancer: A Meta-analysis and a Review of the Mechanisms Involved

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Abstract

Red meat and processed meat intake is associated with a risk of colorectal cancer, a major cause of death in affluent countries. Epidemiological and experimental evidence supports the hypothesis that heme iron present in meat promotes colorectal cancer. This meta-analysis of prospective cohort studies of colon cancer reporting heme intake included 566,607 individuals and 4,734 cases of colon cancer. The relative risk of colon cancer was 1.18 (95% CI: 1.06–1.32) for subjects in the highest category of heme iron intake compared with those in the lowest category. Epidemiological data thus show a suggestive association between dietary heme and risk of colon cancer. The analysis of experimental studies in rats with chemically-induced colon cancer showed that dietary hemoglobin and red meat consistently promote aberrant crypt foci, a putative precancer lesion. The mechanism is not known, but heme iron has a catalytic effect on (i) the endogenous formation of carcinogenic *N*-nitroso compounds and (ii) the formation of cytotoxic and genotoxic aldehydes by lipoperoxidation. A review of evidence supporting these hypotheses suggests that both pathways are involved in heme iron toxicity. *Cancer Prev Res*; 4(2); 177–84. ©2011 AACR.

Introduction

Cancer of the colon and rectum, taken together, are the third most common type of cancer worldwide (1). In most publications, colon and rectal cancer are studied together and the term colorectal cancer (CRC) is used, which we also use here, except when the publications refer specifically to colon or rectal cancer. CRC is the second most common cause of cancer death in affluent countries. Dietary modifications might reduce this cancer burden by up to 70% (2). Three recent meta-analyses showed that total meat intake is not related to risk but that intake of red or processed meat is associated with a modest, but significant risk of CRC (3–5). Processed meat intake appears to be more closely linked with the risk of CRC than fresh red meat intake. In its 2007 report, the World Cancer Research Fund panel recommended that one should limit intake of red meat and avoid processed meat (1).

Several mechanisms may explain the relationship between the risk of CRC and the intake of red or pro-

cessed meat. First, meat cooked at high temperature contains mutagenic heterocyclic amines. But heterocyclic amines might not be major players in CRC risk, as: (i) consumption of chicken is a major contributor to intake of heterocyclic amines, but is not associated with the risk (6); and (ii) doses of heterocyclic amines that induce cancer in animals are 1,000 to 100,000 times higher than the dose ingested by humans (7). A second hypothesis suggests that the high saturated fat content of red and processed meat increases the risk of CRC. But several studies, including a recent meta-analysis, showed no effect of saturated fat on colorectal carcinogenesis (8–11). A third hypothesis concerns the carcinogenic *N*-nitroso compounds (NOC), which can be formed in the gastrointestinal tract by *N*-nitrosation of peptide derived amines or amides. The role of NOC in human cancer is discussed in the following text. Other more unlikely hypotheses involve the high protein, cholesterol, and salt content of red or processed meat. For a review of all these mechanisms, see ref. 12.

Sesink and colleagues suggested that heme iron, in the form of hemin [chloroproporphyrin IX iron(III)] a ferric form of heme, may explain the link between the risk of colon cancer and red meat intake, and the lack of a link with white meat intake (13). Epidemiological and experimental evidence support heme toxicity. Heme consists of an iron atom present at the center of a large heterocyclic organic ring called a porphyrin (Fig. 1). Heme is included in so-called hemoprotein, that is, hemoglobin, myoglobin (both involved in the oxygen supply), and in cytochromes (which catalyze electron transfer reactions). Red meat

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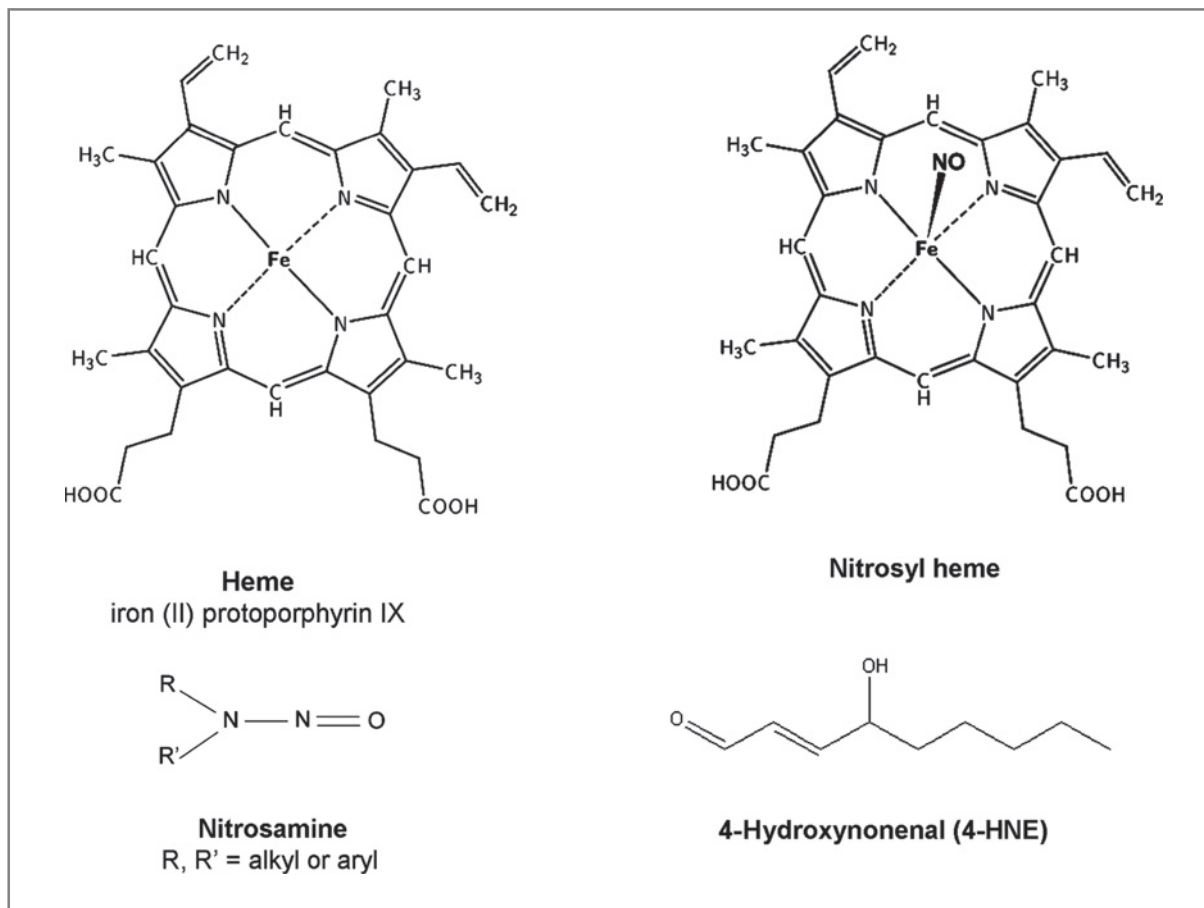


Figure 1. Structure of molecules cited in the review.

(such as beef, veal, lamb, mutton, pork, and offal) owes its dark red color to the presence of a high concentration of myoglobin, and the heme content of red meat is 10-fold higher than that of white meat (such as chicken; ref. 14). In processed red meat, heme iron is nitrosylated, because curing salt contains nitrate or nitrite (Fig. 1; ref. 12).

The aims of the present mini-review were: (i) to conduct a meta-analysis of epidemiological cohort studies on heme intake and the risk of colon cancer; (ii) to review experimental evidence supporting the aforementioned heme hypothesis; and (iii) to understand the mechanism of action of heme in carcinogenesis.

Heme iron intake and risk of colon cancer: a meta-analysis of prospective cohort studies

The objective of this part of the review was to assess, through meta-analysis, the magnitude of the relation between heme iron intake and colon cancer. As most studies do not report data on rectal cancer, we decided to limit our analysis to colon cancer. The methodological procedure is described in the Supplementary Material to this article.

The characteristics of the 5 prospective cohort studies included in the meta-analysis are summarized in Supplementary Data (Table S1). This meta-analysis included data on 566,607 individuals and 4,734 cases of colon cancer. Although 1 cohort study found no association between heme and cancer (15), 3 found that a high intake of heme iron was linked with a higher risk of colon cancer (16–18), and 1 found a positive, but not significant, association between heme iron and colon cancer (19; Fig. 2). In the Lee and colleagues study, the relative risk (RR) for both proximal and distal colon was 1.53 (95% CI: 0.99–2.38). In the Balder and colleagues study, the association was positive in the 2 genders combined (RR = 1.35, 95% CI: 1.03–1.77; ref. 17). The summary RR of colon cancer in all 5 studies was 1.18 (95% CI: 1.06–1.32) for subjects in the highest category of heme iron intake compared with those in the lowest category (Fig. 2). This meta-analysis showed a consistent association between high intake of heme iron and increased risk of colon cancer.

Two studies out of 5 considered calcium in the adjustments for the RR (16–18), and showed the strongest

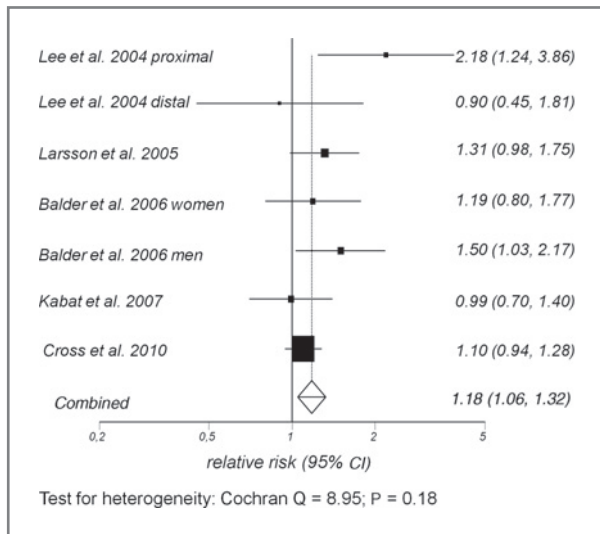


Figure 2. Relative risks of colon cancer in prospective cohort studies, comparing the highest with the lowest category of heme iron consumption. Studies are ordered by year of publication. Squares represent study-specific RR and the size of squares is proportional to the statistical weight that each contributed to the summary estimate of relative risk (percentage weight of each study: Lee *et al.*, 2004, proximal: 6.6%; Lee *et al.*, 2004, distal: 4.6%; Larsson *et al.*, 2005: 18%; Balder *et al.*, 2006, women: 11.7%; Balder *et al.*, 2006, men: 12.86%; Kabat *et al.*, 2007, 14.2%; Cross *et al.*, 2010: 32%). Horizontal lines represent 95% CI. The diamond represents the summary estimate of the relative risk of all studies included in the meta-analysis.

association between heme iron and colon cancer. This makes sense, as calcium inhibits heme-induced cytotoxicity, colonic epithelial hyperproliferation, and promotion of chemically induced carcinogenesis in animal models (20–22).

Two studies we excluded from the meta-analysis found similar results. An ecological study found a direct correlation between the dietary iron index and colon and rectal cancer (23). Ferrucci and colleagues observed a positive, but not significant, association between heme iron in diet and colorectal adenoma.

The present meta-analysis is the first to examine the relation between heme iron and colon cancer. But this study also has its limitations; first it includes only 5 cohort studies, and the way heme intake was measured differs in each study. Lee and colleagues and Larsson and colleagues calculated heme iron content in the diet by applying a factor of 0.4 to the total iron content of all meat items which essentially is reporting an overall red meat effect (16, 18). Balder and colleagues multiplied the heme iron content of each meat item by the mean daily intake of the relevant food item, estimated from the Dutch Food Composition Database (17), but the 2 methods yielded similar results (15). Cross and colleagues developed a new heme iron database based on measured values in conjunction with a detailed meat cooking questionnaire (19).

In conclusion, this meta-analysis showed a significant and consistent but modest increase in the risk of colon cancer associated with high heme iron intake. This study should be pursued by future prospective cohort studies, but this epidemiological result is in line with experimental *in vivo* results detailed in the following text.

Experimental evidence of colorectal cancer promotion by heme iron

Sawa and colleagues showed that dietary hemoglobin produces lipid peroxyl radicals and increases the incidence of nitrosomethylurea-induced colon cancer in rats fed polyunsaturated fat (24). Sesink and colleagues studied the effect of hemin-supplemented diet in non-initiated rats. Dietary hemin increases fat peroxidation and cytotoxic activity of fecal water, and epithelial proliferation by 70% (13). In hemin, the iron atom is stabilized by a freely exchangeable chloride. Pierre and colleagues also showed that hemin and hemoglobin increase the number of azoxymethane-induced aberrant crypt foci, which are putative preneoplastic lesions, in the colon of rats (21). In contrast with hemin, dietary hemoglobin does not increase the cytotoxicity of fecal water, and it is less potent than hemin in promoting colon carcinogenesis. Hemoglobin may be a suitable substitute for myoglobin in nutritional experiments with animal model, and a model agent for studies on the cytotoxicity of red meat (21).

Pierre and colleagues also fed 3 types of meat with different heme content (chicken, beef, and blood sausage) to rats treated with azoxymethane and fed a low-calcium diet (25). This study was the first to show that dietary meat can promote colon carcinogenesis, and that the effect depends on the heme concentration. The results of this study of meat contrast with those of several earlier studies, where red-meat based high-calcium diets failed to promote colon carcinogenesis, indicating probable protection by calcium (26). Subsequently, Pierre and colleagues tested the hypothesis, suggested by epidemiology, that nitrosyl heme in processed meat was more toxic than native heme in fresh meat (27). Cured meat can indeed promote colon carcinogenesis in rats (27). Dietary hemin, but not hemoglobin, could be used as a model agent to mimic the effects of processed meat in rats (27). In a recent study, Pierre and colleagues demonstrated that the nitrosylation of heme was a key event in the promoting effect of processed meat in rats (28).

Analysis of the results of experimental studies of rats with chemically-induced colon cancer (21, 22, 25, 29), showed that the global standardized effect size for number of aberrant crypt foci per colon was 1.73 (95% CI: 1.33–2.14) in rats given dietary heme iron in hemoglobin or beef meat, compared with control rats. The logistic regression approach showed a significant correlation between the number of aberrant crypts per colon and the concentration of heme in the diet ($P = 0.02$; see Methods and Figure in Supplementary Data). This experimental evidence that heme iron promotes carcinogenesis in rats is consistent

with epidemiological evidence. Heme promotion may explain why the intake of red and processed meat is associated with a risk of CRC.

Possible mechanisms of heme toxicity in the gastrointestinal tract

The mechanisms implicated in the promotion of colorectal cancer by heme are poorly understood. The mechanistic hypotheses are based on the catalytic effect of heme iron on (i) the formation of NOC and (ii) the formation of lipid oxidation endproducts.

Heme iron catalyzes N-nitrosation

NOC are formed by N-nitrosation of amines and amides, produced primarily by bacterial decarboxylation of amino acids in the presence of a nitrosating agent (30). There was no *a priori* reason to think that nitrosation would require heme iron. The structure of nitrosamine is shown in Figure 1. NOC can be detected by thermal energy analysis following the release of nitric oxide from biological samples. This analytical procedure comprises nitrosyl iron and S-nitrosothiols in addition to nitrosamines and nitrosamides, which are collectively referred to as apparent total N-nitroso compounds (ATNC; ref. 31).

Animal and human studies. Bacon-fed rats had a fecal concentration of ATNC 10 to 20 times higher than control rats (32). In addition, mice fed a diet of hot-dogs (18%), had 4 to 5 times more ATNC, and mice fed a beef diet had 2 to 3 times more ATNC in their feces than controls fed no meat (33, 34).

Human volunteers given a high red meat diet excreted much more ATNC in their stools than controls given no or little red meat, or only white meat (31, 35, 36). The fecal concentration of ATNC was 60 times higher in volunteers given cured red meat than in volunteers given a vegetarian diet (37). Heme iron, and not inorganic iron or meat proteins, may be responsible for the nitrosation observed in the gut of volunteers fed red meat (38).

Nature of ATNC. A red meat diet increased nitrosyl iron and nitrosothiols in ileal outputs and in stools of volunteers, compared with a vegetarian diet, suggesting that these compounds contribute significantly to ATNC (39, 40). Nitrosothiols are rapidly formed from nitrite and thiol groups at low pH in the stomach and can be precursors for the formation of nitrosyl heme and NOC in the gut (39). The strong correlation between fecal nitrosyl iron and fecal heme suggests that nitrosyl heme is the main source of nitrosyl iron (39). Moreover, ATNC precursors from hot dogs were partially purified and separated by HPLC (41). One fraction was identified as 1-deoxy-N-1-glucosyl glycine by mass spectrometry, and the nitrosated fraction was shown to be mutagenic by the Ames test (41).

Carcinogenicity of nitrosated compounds. The carcinogenicity of ATNC formed in the gut after eating heme from red or processed meat is unknown. Parnaud and colleagues

found no initiation or promotion of preneoplastic lesions by ATNC in the colon of rats fed a bacon-based diet (32). Kunhle and colleagues speculated that nitrosyl iron compounds and nitrosothiols may contribute to the tumorigenic potential of the diet (39). By contrast, in a commentary on Kunhle's article, Hogg speculated that the sequestration of the "nitrosating potential" of the diet as nitrosothiol or as nitrosyl iron may be a protective mechanism that would limit the formation of DNA alkylating agents (42).

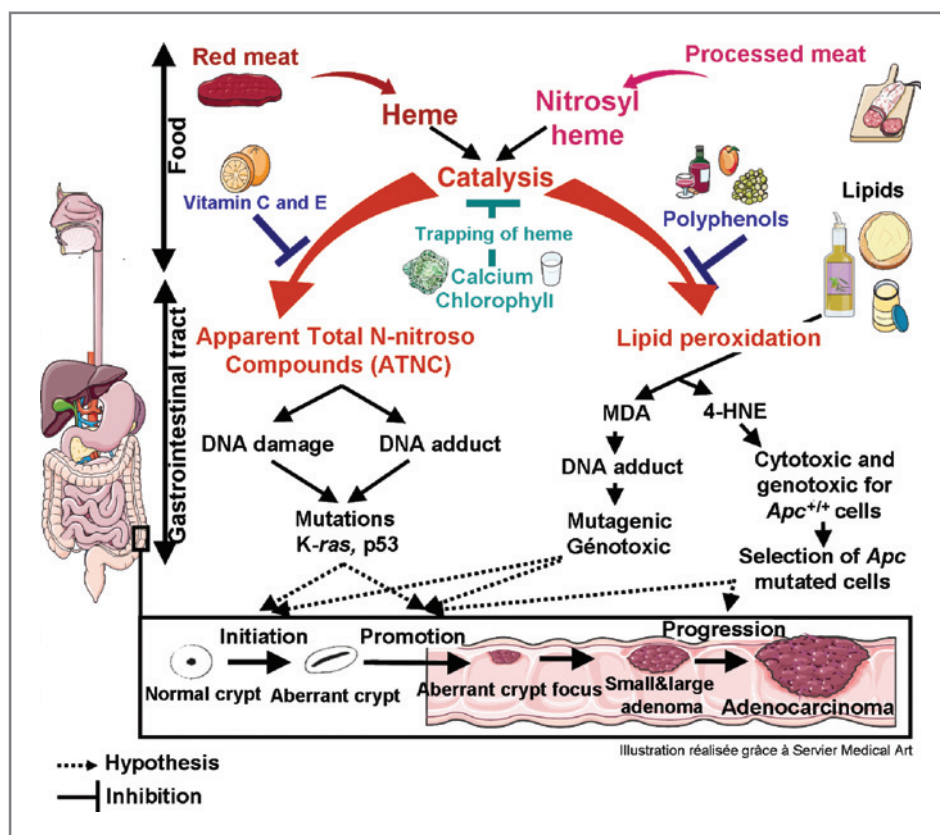
However, several arguments suggest that ATNC may be important genotoxins. First, most NOC, such as nitrosamines, nitrosamides, and nitrosoguanidines, can yield alkylating agents during metabolism, and cause DNA damage. For instance N-methyl-N-nitrosourea intrarectally perfused induced G → A transitions in *K-ras* in 30% of rat colon carcinoma (43). In addition, nitrosated glycine derivatives reacted with DNA to give rise to promutagenic and toxic adducts including O⁶-methylguanine and O⁶-carboxymethylguanine (44). O⁶-Carboxymethylguanine adducts were found in stool exfoliated colonocytes from volunteers eating red meat, with a correlation between the level of adducts and of fecal ATNC, suggesting that ATNC are genotoxic (45). Moreover, potassium diazoacetate, a stable form of nitrosated glycine, was shown to induce mutations in the *p53* gene in a functional yeast assay (46). The patterns of mutations were similar to the patterns observed in human colon tumors. This supports the hypotheses that nitrosation of compounds related to glycine contributes to *p53* mutations in humans, and that O⁶-carboxymethylguanine adducts in exfoliated colorectal cells are related to CRC (46).

Heme iron catalyzes the oxidation of polyunsaturated fats

The polyunsaturated fatty acid residues of phospholipids are extremely sensitive to oxidation. Lipid peroxidation is initiated by free-radical attack of membrane lipids and is catalyzed by heme with the following reaction: LOOH (lipid hydroperoxide) + Fe-ligand (heme) → LOOFe ligands → LO· (lipid alkoxy radical) + ·OFe ligands (heme oxiradical; ref. 47). The initial products of unsaturated fatty acid oxidation are lipid hydroperoxides, but they are relatively short lived. They are either reduced by glutathione peroxidase to unreactive fatty acid alcohols or they react with metals to produce a variety of reactive compounds such as epoxides and aldehydes. The major aldehyde products of lipid peroxidation are malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE; ref. 48). These dietary lipid oxidation end products are risk factors for several human diseases (for review, see refs. 49, 50).

Malondialdehyde. MDA is formed by oxidation of polyunsaturated fatty acids with 2 or more double bonds. MDA-induced DNA damage is mutagenic in bacterial, mammalian, and human cells (51–53). MDA reacts with DNA to form adducts with deoxyguanosine, deoxyadenosine, and deoxycytidine (for review, see ref. 54). The major

Figure 3. Catalytic effects of heme on the formation of ATNC and lipid peroxidation, and their inhibition. Consequences for the development of CRC. Heme catalyzes the formation of ATNC and lipid peroxidation endproducts, which partially explains the promoting effect of red and processed meat on CRC. The catalytic effects of heme can be inhibited by trapping the heme (calcium, chlorophyll). The endogenous formation of ATNC is inhibited by vitamins C and E, and it appears that polyphenols can inhibit lipid peroxidation.



DNA adduct formed by reaction of MDA with DNA is 1,*N*²-malondialdehyde-deoxyguanosine (M₁dG). M₁dG was detected in colorectal biopsies from normal mucosa of 162 participants in the United Kingdom FlexiScope Sigmoidoscopy Screening Trial and the EPIC study (55). The level of this adduct was modulated by dietary and lifestyle habits, and there is to higher M₁dG levels in subjects with adenoma compared with adenoma-free subjects ($P < 0.005$; ref. 55).

4-Hydroxynonenal. In contrast with MDA, 4-HNE is weakly mutagenic but appears to be the main toxic product of lipid peroxidation (Fig. 1). 4-HNE has powerful effects on signal transduction pathways and some of its effects appear to be independent of DNA damage (48). Indeed, 4-HNE present in fecal water can induce apoptosis and necrosis of human colon carcinoma cells through caspase 3 activation (56). Mutations in the *adenomatous polyposis coli* (*Apc*) gene on the chromosome 5q21 locus are considered to be one of the earliest events in the initiation of CRC (57). Moreover, *Apc* mutation was shown to reduce the level of caspases 3, 7, and 9 in mouse colonocytes, leading to resistance to apoptosis (58). An intestinal cell line derived from C57BL/6J mice (*Apc*^{+/+}) and Min mice (*Apc* Min^{+/+}) retained the heterozygous *Apc* genotype and the disordered actin cytoskeleton network for the *Apc* Min^{+/+} cell line (59, 60). By

exposing this cell line to fecal water of heme-fed rats or to 4-HNE, Pierre and colleagues showed that apoptosis was suppressed in *Apc* Min^{+/+} cells (61). The heterozygote *Apc* mutation is thus a strong selective advantage for colonic cells exposed to a lipoperoxidation-related genotoxic environment such as excess heme iron or 4-HNE (61).

In summary, heme catalyzes the formation of ATNC and of lipid oxidation end products, which may explain the promoting effects of red and processed meat on CRC. However, the procarcinogenic effect of heme can be inhibited by several molecules. First, calcium salts and chlorophyll can precipitate heme molecules and inhibit the cytotoxic and hyperproliferative effect of heme in the rat epithelium (17, 20–22, 62, 63). Moreover, the endogenous formation of ATNC is inhibited by vitamins C and E, and lipoperoxidation is inhibited by several polyphenols such as quercetin, α -tocopherol, or red wine polyphenols (64–68). The catalytic effects of heme and its inhibition are summarized in Figure 3.

Conclusion

CRC is the leading cause of cancer death among non-smokers in affluent countries, and its prevention is thus a major goal for public health. Epidemiological studies

demonstrate a modest but significant and consistent relation between red meat and processed meat intake and CRC risk. The dietary recommendations are to reduce red meat intake and to avoid processed meat intake (1). However, meat is an important source of proteins, providing all essential amino acids, and it is an excellent source of iron and zinc. Iron deficiency is the most widespread nutritional disorder in the world, especially among children and premenopausal women, and results in iron deficiency anemia (1). Knowledge of the mechanism of CRC promotion by meat may allow an alternative prevention strategy to be developed: inhibiting red and processed meat toxicity instead of stopping meat intake. Among the hypotheses explaining the association between meat intake and the risk of CRC, the effect of heme iron is supported by both epidemiological (Fig. 2) and experimental evidence (Supplementary Fig. S1). Several mechanisms may explain the effect of heme on CRC, and the 2 major hypotheses are: (i) heme catalyzes the endogenous formation of ATNC; and (ii) heme catalyzes the peroxidation of dietary fats (Fig. 3). Calcium salts, chlorophyll, vitamin C, and several polyphenols may

reduce these deleterious effects of heme. Specific recommendations might be made, for example, "eat a yogurt after your steak." Moreover, vitamins or polyphenols could be added during the curing process. Ascorbic acid is already added during the processing of processed meats specifically to inhibit the formation of volatile NOC in the meat (69). We expect that this will reduce the risk of CRC without losing the benefit and the pleasure of eating meat.

Disclosure of Potential Conflicts of Interests

No potential conflicts of interests were disclosed.

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References

1. WCRF. Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective. Washington, DC: WCRF and American Institute for Cancer Research; 2007. p. 1-537.
2. Cummings JH, Bingham SA. Diet and the prevention of cancer. *BMJ* 1998;317:1636-40.
3. Sandhu MS, White IR, McPherson K. Systematic review of the prospective cohort studies on meat consumption and colorectal cancer risk: a meta-analytical approach. *Cancer Epidemiol Biomarkers Prev* 2001;10:439-46.
4. Norat T, Lukanova A, Ferrari P, Riboli E. Meat consumption and colorectal cancer risk: dose-response meta-analysis of epidemiological studies. *Int J Cancer* 2002;98:241-56.
5. Larsson SC, Wolk A. Meat consumption and risk of colorectal cancer: a meta-analysis of prospective studies. *Int J Cancer* 2006;119:2657-64.
6. Sinha R, Rothman N, Brown ED, Mark SD, Hoover RN, Caporaso NE, et al. Pan-fried meat containing high levels of heterocyclic aromatic amines but low levels of polycyclic aromatic hydrocarbons induces cytochrome p4501a2 activity in humans. *Cancer Res* 1994;54:6154-9.
7. Stavric B. Biological significance of trace levels of mutagenic heterocyclic aromatic amines in human diet: a critical review. *Food Chem Toxicol* 1994;32:977-94.
8. Nauss KM, Lochniskar M, Newberne PM. Effect of alteration in the quality and quantity of dietary fat on DMH-induced colon tumorigenesis in rats. *Cancer Res* 1983;43:4083-90.
9. Nutter RL, Gridley DS, Kettering JD, Goude AG, Slater JM. BALB/c mice fed milk or beef protein: differences in response to 1,2-dimethylhydrazine carcinogenesis. *J Natl Cancer Inst* 1983;71:867-74.
10. Clinton SK, Imrey PB, Mangian HJ, Nandkumar S, Visek WJ. The combined effects of dietary fat, protein, and energy intake on azoxymethane-induced intestinal and renal carcinogenesis. *Cancer Res* 1992;52:857-65.
11. Alexander DD, Cushing CA, Lowe KA, Scurman B, Roberts MA. Meta-analysis of animal fat or animal protein intake and colorectal cancer. *Am J Clin Nutr* 2009;89:1402-9.
12. Santarelli RL, Pierre F, Corpet DE. Processed meat and colorectal cancer: a review of epidemiologic and experimental evidence. *Nutr Cancer* 2008;60:131-44.
13. Sesink ALA, Termont DSML, Kleibeuker JH, Vandermeer R. Red meat and colon cancer: the cytotoxic and hyperproliferative effects of dietary heme. *Cancer Res* 1999;59:5704-9.
14. Schwartz S, Ellefson M. Quantitative fecal recovery of ingested hemoglobin-heme in blood: comparisons by HemoQuant assay with ingested meat and fish. *Gastroenterology* 1985;89:19-26.
15. Kabat GC, Miller AB, Jain M, Rohan TE. A cohort study of dietary iron and heme iron intake and risk of colorectal cancer in women. *Br J Cancer* 2007;97:118-22.
16. Larsson SC, Adami HO, Giovannucci E, Wolk A. Re: Heme iron, zinc, alcohol consumption, and risk of colon cancer. *J Natl Cancer Inst* 2005;97:232-3.
17. Balder HF, Vogel J, Jansen MC, Weijenberg MP, Van den Brandt PA, Westenbrink S, et al. Heme and chlorophyll intake and risk of colorectal cancer in the Netherlands cohort study. *Cancer Epidemiol Biomarkers Prev* 2006;15:717-25.
18. Lee DH, Anderson KE, Harnack LJ, Folsom AR, Jacobs DR Jr. Heme iron, zinc, alcohol consumption, and colon cancer: Iowa Women's Health Study. *J Natl Cancer Inst* 2004;96:403-7.
19. Cross AJ, Ferrucci LM, Risch A, Graubard BI, Ward MH, Park Y, et al. A large prospective study of meat consumption and colorectal cancer risk: an investigation of potential mechanisms underlying this association. *Cancer Res* 2010;70:2406-14.
20. Sesink ALA, Termont DSML, Kleibeuker JH, VanDerMeer R. Red meat and colon cancer: dietary haem-induced colonic cytotoxicity and epithelial hyperproliferation are inhibited by calcium. *Carcinogenesis* 2001;22:1653-9.
21. Pierre F, Tache S, Petit CR, Van Der Meer R, Corpet DE. Meat and cancer: haemoglobin and haemin in a low-calcium diet promote colorectal carcinogenesis at the aberrant crypt stage in rats. *Carcinogenesis* 2003;24:1683-90.
22. Pierre F, Santarelli R, Tache S, Gueraud F, Corpet DE. Beef meat promotion of dimethylhydrazine-induced colorectal carcinogenesis

- biomarkers is suppressed by dietary calcium. *Br J Nutr* 2008;99:1000–6.
23. Grant WB. An ecological study of cancer mortality rates including indices for dietary iron and zinc. *Anticancer Res* 2008;28:1955–63.
 24. Sawa T, Akaike T, Kida K, Fukushima Y, Takagi K, Maeda H. Lipid peroxyl radicals from oxidized oils and heme-iron: implication of a high-fat diet in colon carcinogenesis. *Cancer Epidemiol Biomarkers Prev* 1998;7:1007–12.
 25. Pierre F, Freeman A, Tache S, Van Der Meer R, Corpet DE. Beef meat and blood sausage promote the formation of azoxymethane-induced mucin-depleted foci and aberrant crypt foci in rat colons. *J Nutr* 2004;134:2711–6.
 26. Parnaud G, Peiffer G, Tache S, Corpet DE. Effect of meat (beef, chicken, and bacon) on rat colon carcinogenesis. *Nutr Cancer* 1998;32:165–73.
 27. Pierre FH, Santarelli RL, Allam O, Tache S, Naud N, Gueraud F, et al. Freeze-dried ham promotes azoxymethane-induced mucin-depleted foci and aberrant crypt foci in rat colon. *Nutr Cancer* 2010;62:567–73.
 28. Santarelli RL, Vendevre JL, Naud N, Tache S, Gueraud F, Viau M, et al. Meat processing and colon carcinogenesis: cooked, nitrite-treated, and oxidized high-heme cured meat promotes mucin-depleted foci in rats. *Cancer Prev Res* 2010;3:852–64.
 29. Belobrajdic DP, McIntosh GH, Owens JA. Whey proteins protect more than red meat against azoxymethane induced ACF in Wistar rats. *Cancer Lett* 2003;198:43–51.
 30. Mirvish SS. Role of *N*-nitroso compounds (NOC) and *N*-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. *Cancer Lett* 1995;93:17–48.
 31. Hughes R, Cross AJ, Pollock JRA, Bingham S. Dose-dependent effect of dietary meat on endogenous colonic *N*-nitrosation. *Carcinogenesis* 2001;22:199–202.
 32. Parnaud G, Pignatelli B, Peiffer G, Tache S, Corpet DE. Endogenous *N*-nitroso compounds, and their precursors, present in bacon, do not initiate or promote aberrant crypt foci in the colon of rats. *Nutr Cancer* 2000;38:74–80.
 33. Haorah J, Mirvish SS. Determination of total *N*-nitroso compounds and their precursors in Franfurters, fresh meat, dried salted fish, sauces, tobacco, and tobacco smoke particulates. *J Agric Food Chem* 2001;49:6068–78.
 34. Mirvish SS, Haorah J, Zhou L, Hartman M, Morris CR, Clapper ML. *N*-Nitroso compounds in the gastrointestinal tract of rats and in the feces of mice with induced colitis or fed hot dogs or beef. *Carcinogenesis* 2003;24:595–603.
 35. Bingham SA, Pignatelli B, Pollock JRA, Ellul A, Malaveille C, Gross G, et al. Does increased endogenous formation of *N*-nitroso compounds in the human colon explain the association between red meat and colon cancer? *Carcinogenesis* 1996;17:515–23.
 36. Bingham SA, Hughes R, Cross AJ. Effect of white versus red meat on endogenous *N*-nitrosation in the human colon and further evidence of a dose response. *J Nutr* 2002;132:3522S–5S.
 37. Joosen AM, Kuhnle GG, Aspinall SM, Barrow TM, Lecommandeur E, Azqueta A, et al. Effect of processed and red meat on endogenous nitrosation and DNA damage. *Carcinogenesis* 2009;30:1402–7.
 38. Cross AJ, Pollock JRA, Bingham SA. Haem, not protein or inorganic iron, is responsible for endogenous intestinal *N*-nitrosation arising from red meat. *Cancer Res* 2003;63:2358–60.
 39. Kuhnle GG, Bingham SA. Dietary meat, endogenous nitrosation and colorectal cancer. *Biochem Soc Trans* 2007;35:1355–7.
 40. Lunn JC, Kuhnle G, Mai V, Frankenfeld C, Shuker DE, Glen RC, et al. The effect of haem in red and processed meat on the endogenous formation of *N*-nitroso compounds in the upper gastrointestinal tract. *Carcinogenesis* 2007;28:685–90.
 41. Zhou L, Haorah J, Perini F, Carmella SG, Shibamoto T, Mirvish SS. Partial purification from hot dogs of *N*-nitroso compound precursors and their mutagenicity after nitrosation. *J Agric Food Chem* 2006;54:5679–87.
 42. Hogg N. Red meat and colon cancer: heme proteins and nitrite in the gut. A commentary on "diet-induced endogenous formation of nitroso compounds in the GI tract". *Free Radic Biol Med* 2007;43:1037–9.
 43. Jacoby RF, Alexander RJ, Raicht RF, Brasitus TA. K-ras oncogene mutations in rat colon tumors induced by *N*-methyl-*N*-nitrosourea. *Carcinogenesis* 1992;13:45–9.
 44. Shuker DE, Margison GP. Nitrosated glycine derivatives as a potential source of O⁶-methylguanine in DNA. *Cancer Res* 1997;57:366–9.
 45. Lewin MH, Bailey N, Bandaletova T, Bowman R, Cross AJ, Pollock J, et al. Red meat enhances the colonic formation of the DNA adduct O⁶-carboxymethyl guanine: implications for colorectal cancer risk. *Cancer Res* 2006;66:1859–65.
 46. Gottschalg E, Scott GB, Burns PA, Shuker DE. Potassium diazoacetate-induced p53 mutations *in vitro* in relation to formation of O⁶-carboxymethyl- and O⁶-methyl-2'-deoxyguanosine DNA adducts: relevance for gastrointestinal cancer. *Carcinogenesis* 2007;28:356–62.
 47. Tappel A. Heme of consumed red meat can act as a catalyst of oxidative damage and could initiate colon, breast and prostate cancers, heart disease and other diseases. *Med Hypotheses* 2007;68:562–4.
 48. Marnett LJ. Oxyradicals and DNA damage. *Carcinogenesis* 2000;21:361–70.
 49. Kanner J. Dietary advanced lipid oxidation endproducts are risk factors to human health. *Mol Nutr Food Res* 2007;51:1094–101.
 50. Corpet DE, Gueraud F, O'Brien P. Heme iron from dietary meat produces pro-carcinogenic peroxides endogenously. In: Bruce WR, O'Brien P, editors. *Endogenous Toxins: Diet, Genetics, Disease and Treatment*. Wiley-VCH; 2010. p. 133–49.
 51. Basu AK, Marnett LJ. Unequivocal demonstration that malondialdehyde is a mutagen. *Carcinogenesis* 1983;4:331–3.
 52. Yau TM. Mutagenicity and cytotoxicity of malonaldehyde in mammalian cells. *Mech Ageing Dev* 1979;11:137–44.
 53. Niedernhofer LJ, Daniels JS, Rouzer CA, Greene RE, Marnett LJ. Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. *J Biol Chem* 2003;278:31426–33.
 54. Marnett LJ. Lipid peroxidation-DNA damage by malondialdehyde. *Mutat Res* 1999;424:83–95.
 55. Leuratti C, Watson MA, Deag EJ, Welch A, Singh R, Gottschalg E, et al. Detection of malondialdehyde DNA adducts in human colorectal mucosa: relationship with diet and the presence of adenomas. *Cancer Epidemiol Biomarkers Prev* 2002;11:267–73.
 56. Awasthi YC, Sharma R, Cheng JZ, Yang Y, Sharma A, Singhal SS, et al. Role of 4-hydroxynonenal in stress-mediated apoptosis signaling. *Mol Aspects Med* 2003;24:219–30.
 57. Powell SM, Zilz N, Beazer-Barclay Y, Bryan TM, Hamilton SR, Thibodeau SN, et al. APC mutations occur early during colorectal tumorigenesis. *Nature* 1992;359:235–7.
 58. Chen T, Yang I, Irby R, Shain KH, Wang HG, Quackenbush J, et al. Regulation of caspase expression and apoptosis by adenomatous polyposis coli. *Cancer Res* 2003;63:4368–74.
 59. Pierre F, Perrin P, Bassonga E, Bornet F, Meflah K, Menanteau J. T cell status influences colon tumor occurrence in min mice fed short chain fructo-oligosaccharides as a diet supplement. *Carcinogenesis* 1999;20:1953–6.
 60. Forest V, Pierre F, Bassonga E, Meflah K, Olivier C, Menanteau J. Apc^{+/min} colonic epithelial cells express TNF receptors and ICAM-1 when they are co-cultured with large intestine intra-epithelial lymphocytes. *Cell Immunol* 2003;223:70–6.
 61. Pierre F, Tache S, Gueraud F, Rerole AL, Jourdan ML, Petit C. Apc mutation induces resistance of colonic cells to lipoperoxide-triggered apoptosis induced by faecal water from haem-fed rats. *Carcinogenesis* 2007;28:321–7.
 62. de Vogel J, Jonker-Termont DS, van Lieshout EM, Katan MB, Van Der Meer R. Green vegetables, red meat and colon cancer: chlorophyll prevents the cytotoxic and hyperproliferative effects of haem in rat colon. *Carcinogenesis* 2005;26:387–93.
 63. de Vogel J, Jonker-Termont DS, Katan MB, Van Der Meer R. Natural chlorophyll but not chlorophyllin prevents heme-induced cytotoxic

- and hyperproliferative effects in rat colon. *J Nutr* 2005;135:1995–2000.
64. Mirvish SS. Effects of vitamins C and E on *N*-nitroso compound formation, carcinogenesis, and cancer. *Cancer* 1986;58:1842–50.
65. Douglass ML, Kabacoff BL, Anderson GA, Cheng MC. The chemistry of nitrosamine formation, inhibition and destruction. *J Soc Cosmet Chem* 1978;29:581–606.
66. Ross JA, Kasum CM. Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annu Rev Nutr* 2002;22:19–34.
67. Vulcain E, Goupy P, Caris-Veyrat C, Dangles O. Inhibition of the metmyoglobin-induced peroxidation of linoleic acid by dietary antioxidants: action in the aqueous vs. lipid phase. *Free Radic Res* 2005;39:547–63.
68. Gorelik S, Ligumsky M, Kohen R, Kanner J. A novel function of red wine polyphenols in humans: prevention of absorption of cytotoxic lipid peroxidation products. *FASEB J* 2008;22:41–6.
69. Mirvish SS. Blocking the formation of *N*-nitroso compounds with ascorbic acid *in vitro* and *in vivo*. *Ann N Y Acad Sci* 1975;258:175–80.