Review Article

Effect of diet and gut environment on the gastrointestinal formation of *N*-nitroso compounds: a review

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Abstract

Diet is associated with the development of cancer in the gastrointestinal (GI) tract, because dietary nitrate and nitrite are the main nitrosating agents that are responsible for the formation of carcinogenic *N*-nitroso compounds (NOCs) when nitrosatable substrates, such as amine and amide, are present in the GI tract. However, whether the nitroso compounds become beneficial *S*-nitroso compounds or carcinogenic NOCs might depend on dietary and environmental factors including food stuffs, gastric acidity, microbial flora, and the mean transit time of digesta. This review focused on GI NOC formation and environmental risk factors affecting its formation to provide appropriate nutritional strategies to prevent the development of GI cancer.

1. Introduction[1]

Most gastrointestinal (GI) cancers are sporadic and arise in individuals with environmental rather than hereditary risk factors. Among the environmental factors influencing the risk of developing GI cancer, diet is the strongest contributor [88]. Western diets, which are typified by high fat, high meat, and low fiber content, are associated with an increased risk of colorectal cancers. A high-fat diet increases bile acid secretion that is transformed by colonic microbiota into secondary bile acid with genotoxic properties of DNA damage due to reactive oxygen and nitrogen species [7,87]. On the other hand, a high fiber diet leads to undigested carbohydrate residue in the colon, which is fermented into short chain fatty acids due to obligated anaerobic bacteria residing in the lower intestine. These bacteria provide not only a major energy-yielding substrate for epithelial cells, but also beneficial intestinal environments that suppress inflammatory responses and protect against cancer development [33,52]. On the other hand, protein-rich diets provide inflammatory and toxic nitrogenous metabolites such as phenols, indoles, ammonia, and amines that are provided by microbial fermentation of undigested protein residues [38]. These nitrogenous metabolites include *N*-nitroso compounds (NOCs), such as nitrosamine and nitrosamide, which are well-known potential carcinogens formed by the reaction of nitrosating agents, such as nitrite and secondary amines and amides, and are a prominent risk factor of GI cancer. They are potent alkylating agents that induce G-C to A-T transitions at the second base of codon 12 or 13 of the K-*ras* gene [11] in the epithelial cells, and cause cancer development in the GI tract.

Many studies of mice and rats that were given nitrite in their food and drinking water showed increased incidences of benign and malignant tumors at many organ sites [29,54], providing sufficient evidence of the GI carcinogenicity of nitrite [30]. On the other hand, nitrate itself is relatively non-toxic below maximum levels in the context of carcinogenicity. However, the catalytic intermediates of nitrate, such as N_2O_3 and NO^+, are important in NOC formation and carcinogenesis. Their presence has led to the present restrictions on nitrate in drinking water and the current acceptable daily intake recommendations of nitrate and nitrite issued by the European Food Safety Authority and the World Health Organization (WHO) [2,83]. According to the Joint Food and Agriculture Organization/WHO Expert Committee on Food Additives in 2008, epidemiological studies showed no consistently increased risk for cancer with increasing consumption of nitrate. FESA also stated in its 2008 report that epidemiological studies do not suggest that nitrate intake from diet or drinking water is associated with increased cancer risk [2]. In epidemiological studies on humans, although chronic exposure to nitrate in food and drinking water was reported to be associated with an increased risk of colon cancer, its risk was limited to those with low vitamin C intake and high meat intake [24,25], suggesting that its risk is likely to be affected by a combination of food and dietary nitrate.

Dietary nitrate and nitrite usually come from vegetables and fruits, and are experimentally and

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[1] Abbreviations: GI: gastrointestinal; NOC: *N*-nitroso compounds; IBD: inflammatory bowel disease; pO_2: partial oxygen pressure

epidemiologically demonstrated to be protective against cancer development because of the many nitrosation inhibitors that are included in these foods [13]. In addition, saliva is a major dietary source of nitrite, and it is always swallowed with fully masticated foods. Around 93% of the total daily ingestion of nitrite is from saliva [4,14], because the enterosalivary route provides nitrite by recycling 25% of the dietary nitrate in the oral cavity [64], where salivary nitrate is reduced to nitrite by oral bacteria. It then enters the stomach. If this nitrite is a main contributor to NOC formation and subsequent cancer promotion, it is necessary to keep spitting to expel the saliva. However, clinically, this is not necessarily the case. This is because NOC formation in the GI tract is multifacetedly affected by a combination of many environmental factors including a variety of nitrosating agents, food stuffs, gastric acidity, and intestinal microbial flora. Therefore, a question arises as to what drives dietary nitrate toward the promoter or protector of GI tract cancer. This review focused on carcinogenic NOC formation and the environmental risk factors affecting its formation, and provided an appropriate scientific approach to nutritional strategies to prevent GI tract cancer.

2. *N*-nitroso compounds and gastrointestinal cancer

Dietary intake of preformed NOCs, which are included in cured and processed meat and beer, is positively associated with colorectal cancer [55], but endogenous NOCs can be formed more often wherever both nitrosating agents and nitrosatable substrates coexist in the body [38]. This formation in the GI tract is affected by many factors including diet such as red meat, with or without dietary antioxidants such as polyphenol and ascorbic acid [59], stomach acidity [32], medication with antacids such as a proton pump inhibitor [59], bacterial flora in the gut, and the mean transit time of dietary residue through the colon [39] (Table 1). Below, we will propose three mechanisms for this process: chemical (acid-catalyzed), bacterial, and inflammatory NOC formations, and discuss in detail the underlying factors affecting NOC formation in the GI tract in the esophagus, stomach, and colon.

3. *N*-nitroso compounds in the esophagus and stomach

The stomach might be a catalytic organ that drives dietary nitrate and nitrite toward beneficial or carcinogenic NO-related compounds. In general, dietary nitrite, the major nitrosating agent derived from diets and/or reduction of salivary nitrate due to oral bacteria, is catalyzed in the acidic stomach to generate NO-related compounds, such as *S*-nitroso, N-nitroso, *O*-nitroso compounds, and NO [28,67]. In the acidic stomach, nitrite equilibrates with nitrous acid (pK_a value of 3.3-3.4); for example, in gastric fluid with a pH of 2, most of the nitrite in the stomach will be present in a protonated form as HNO_2, which releases nitrosonium ion (NO^+), which is a potent nitrosating species, via N_2O_3. Protein thiols (RS-H), amines (RN-H), and phenol groups (RO-H) are primary substrates for *S*-, *N*-, and *O*-nitrosation with NO^+ via an electrophilic attack on these organic compounds, leading to the formation of *S*-nitrosothiol (RS-NO), *N*-nitrosamine (RN-NO), and *O*-nitroso compounds, such as ethyl nitrite, respectively. Although NOC formation in the GI tract

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might depend on dietary nitrate and nitrite [71], gastric *N*-nitrosation level is reported to be ten times lower than fecal levels in healthy subjects [66], because amines and phenol groups are poorer nucleophiles than sulfur atoms. Low pH in the stomach kinetically favors the formation of *S*-nitrosothiol rather than *N*-nitrosamine [20] (Fig. 1). In addition, the *N*-nitrosation reaction is inhibited by appreciable amounts of ascorbic acid that are secreted in gastric juice [79] and concurrent ingestion of fruits and vegetables that are rich in antioxidants such as polyphenols and vitamins C and E (by promoting NO generation from nitrite) [5,35,86]. An *in vitro* study also showed the inhibitory effects of onion and garlic, which are rich in sulfhydryl compounds, on the formation of NOC [45,77].

    S-nitrosothiol systemically transduces NO bioactivity by acting as a relatively stable NO donor or transnitrosating agent, providing beneficial effects on cardiovascular and metabolic disorders [45]. Recent evidence has shown that reduced NO bioavailability is a critical event that leads to low-grade inflammatory state in the vascular and adipose tissues, resulting in atherosclerosis and insulin resistance [63,82]. Therefore, dietary nitrate and nitrite improve these conditions by restoring NO bioavailability via the enterosalivary nitrate-nitrite-NO pathway [90], which could be more enhanced by concurrent ingestion of antioxidants such as tempol, quercetin, and epicatechin with subsequent vascular benefits [3,56]. Compared with stable *S*-nitrosothiol, NO, which is another dietary nitrite-derived product in the stomach, is a short-lived gaseous molecule, which itself does not directly nitrosate organic molecules. Instead, it increases local mucosal blood flow via a cyclic GMP-dependent mechanism and subsequent mucus thickness, and protects the stomach from inflammation and gastric cancer promotion [45] (Table 1). Recent epidemiological evidence suggests that intake of vegetables and fruits that are rich in nitrate and antioxidants protect against most cancers in the upper and lower GI tracts as well as in the larynx, pancreas, breast, and bladder. Therefore, in the healthy acidic stomach, dietary nitrite favors the catalyzed acid formation of protein *S*-nitrosation (RS-NO) and NO production, which deliver beneficial NO-mediated signals systemically and locally, respectively, rather than producing carcinogenic *N*-nitrosation. Ethyl nitrite, a potent smooth muscle relaxant, is also generated by the interaction of salivary-derived nitrite and ethanol in the acidic stomach [28,90]. This organic nitrite has been studied as a potent nitrosating agent and vasodilator related to physiological and pathophysiological consequences [90].

    Because NO is known to be a molecule with both benefits and deficits, too much NO production may be toxic and carcinogenic and lead to the generation of reactive oxygen species and reactive nitrogen species. For example, maximal NO formation is detected at the gastroesophageal junction, where NO autoxidizes in the cells to form nitrosamine, possibly leading to a high incidence of cancer development at this anatomical site [41].

    On the other hand, gastric atrophy following chronic *Helicobacter pylori* infection and administration of a proton pump inhibitor can lead to an increased pH in the stomach. Acid-catalyzed nitrite disproportionation is diminished, followed by bacterial overgrowth in a hypo- or achlorhydric stomach with very low gastric concentrations of ascorbic acid [79], which forms *N*-nitrosamine from

nitrite by bacterial nitrite reductases. These conditions could favor the formation of *N*-nitrosamine rather than *S*-nitrosothiol [19,50], allowing for a high incidence of gastric cancer development [72].

4. Chemical formation of *N*-nitroso compounds in the colon

In general, most dietary nitrate and nitrite are absorbed early in the upper GI tract, followed by excretion of 75% of absorbed nitrate in the urine; however, the other 25% of the circulating nitrate is recovered in the salivary gland and secreted in the oral cavity. It is then reduced to nitrite and catalyzed into other NO-related compounds, most of which are absorbed in the stomach and upper small intestine before they reach the large intestine [27].

However, for people who consume a large amount of red meat, nitrosating agents travel through the colon via heme-mediated transportation, which begins with acid-catalyzed thionitrosation (Cysteine-NO) of digested proteins and the heme protein itself in the stomach [21]. These thiol groups become more unstable and susceptible to Cu^{2+}-catalyzed decomposition to disulfide formation and NO release, and facilitate the nitrosylation of heme (Fe-NO) by salvaging the released NO when it is passed through the reductive and anaerobic intestine and colon [36] (Fig. 1) (Fe-NO>RS-NO in the feces of a person with a red meat diet, Table 2). Compared with inorganic iron [22], nitrosyl heme is known to act as nitrosating agent for the amines that are available from microbial fermentation of undigested protein residue [49,59]. The *in vitro* experiments by Lunn et al. demonstrated that the level of acidified and neutralized heme, equivalent to that of the stomach and the lower intestine, enhances the nitrosation of amine morpholine, suggesting that gastric acid-catalyzed nitrosothiols were initially formed, followed by intestinal formation of *S*-nitrosyl heme (hemoglobin and myoglobin). This process might be responsible for NOC formation in the lower intestine, even in the presence of minimal microbial flora [8,44,47,57]. Because heme iron is much more abundant in red meat than in white meat and fish, recent studies on humans have clearly indicated that red meat is directly and dose-dependently associated with colonal NOC formation and subsequent colonic formation of the NOC-specific DNA adduct (DNA adduct O^6-carboxymethyl guanine) that causes colorectal cancer [12,53] (Table 2). In addition to contributing to NOC formation, heme iron was reported in a rat experiment to have peroxidase activity and generated carcinogenic lipid peroxidation end-products such as aldehyde [6], suggesting that heme iron-mediated lipid peroxidation could be another important mechanism to promote colorectal cancer [15,76].

Although nitrosyl heme that is formed after red meat consumption is delivered as a nitrosating agent to the small intestine, nitrosyl heme formation occurs much less in the upper GI tract than in the colon, as mentioned above [47]. In addition, because nitrosatable substrates are not formed due to the lack of microbial fermentation of protein residue in the small intestine, NOC formation might be difficult, reasonably suggesting that the incidence of the development of cancer in the small intestine was low [37]. Fruits and vegetables that are rich in fibers and antioxidants appear to reduce the enhanced fecal NOC formation in a person with a red meat diet, and they reduce intestinal transit

time and provide both antioxidants and plant-derived polysaccharides that are digested into short chain fatty acids that maintain normal intestinal microbial flora and the gut health of humans (Table 2). Therefore, many factors that are present in the diet could modify the process of NOC formation and levels of NOC chemical production by acting as catalysts or inhibitors. On the other hand, the intake of meat proteins at a recommended level may increase *Lactobacillus* in the colon and benefit gut health [93]. Nutritionally balanced diets rather than extreme vegan diets are recommended for overall health and weight management. The foods, nutrients, and medicines that affect NOC formation in the GI tract are listed in Table 1.

5. Bacterial formation of *N*-nitroso compounds in the colon

The population of microbes in the intestine increases along the GI tract, reaching the highest population of 10^{12} cfu/g in the feces in the colon [31]. Experimental studies indicated that significantly higher levels of NOCs were detected in the gut contents of rats with conventional microbial flora compared to those who were germ free, suggesting the important role of the microbiota in the formation of NOCs and subsequent development of colorectal cancer [26,58,80, 81,89]. *In vivo* studies have had difficulty identifying the bacteria that are responsible for NOC formation, and a clear cause and effect relationship between intestinal microbiota and colorectal cancer remains to be clarified. However, a large number of *in vitro* incubation studies have demonstrated that NOC forms due to individual human intestinal microbiota (Table 3). Most of these microbiota belong to facultative anaerobes that can also use nitrate or nitrite for respiratory denitrification by reducing nitrate to nitrite, NO, N_2O, and N_2. In general, although most nitrate and nitrite are supposed to be absorbed in the upper GI tract before reaching the lower intestine, in human studies [22,47], nitrite, which is a potent nitrosating agent, is actually present in the contents of the feces, particularly in those of people with a high intake of red meat compared with those with diets of white meat, fish, and vegetables (Table 2). This suggests that the NO-carrying heme protein that reaches the large intestine might be associated with oxidation to nitrite in the colon [8]. Although the precise mechanism remains unknown, close contact between fecal nitrosyl heme and oxygen that diffuse from the enteral capillary vasculature to the lumen could result in nitrite and nitrate formation in the colon's aerobic inner mucous layer, in which oxygen-tolerant facultative anaerobes reside [1]. The anaerobes can switch from aerobic metabolism to nitrate and nitrite-driven anaerobic respiration using nitrate and nitrite reductases [46] that form NOC during respiratory denitrification. In addition, these nitrate and nitrite reductases integrate iron into the enzyme activity. Rats harboring human fecal bacterial flora in the intestine showed a three-fold increase in fecal nitrate reductase activity with a three-fold increase in meat consumption [73].

One of the nitrite reductases that is responsible for NOC formation is cytochrome cd_1-nitrite reductase, and it is the enzyme that catalyzes nitrosation through producing NO or NO^+-like species. *Pseudomonas aeruginosa* and *Enterobacteria* such as *Escherichia coli* and *Proteus morganii* are reported to have this enzyme activity [19]. Nitrate and nitrite serve as electron acceptors to generate

energy for these anaerobic bacteria, allowing them to thrive within a bacterial community. In healthy ecosystems, more than 99% of the bacteria in the lower intestine is occupied by obligate anaerobic bacteria that lack a way to use nitrate and nitrite as electron acceptors; however, dietary nitrate and nitrite without antioxidants and nitrosation inhibitors could reach the lower intestine and grow bacteria that can use nitrate and nitrite to generate NOCs in the colon.

Compared with plant-based diets, animal-based diets affect the intestinal microbial composition due to increases in bile-tolerant microbiota such as *Bacteroides* and decreases in plant fiber-degrading microbiota (butyrate producers) such as *Firmicutes* at the phylum level. However, the effect of red meat in the diet (heme) on the intestinal microbial composition has not yet been established because studies on humans have included small numbers of patients with sampling heterogeneity and individual diversity. However, recent animal studies analyzing the operational taxonomic unit, which is an operational definition that is used to classify groups of closely related individuals, demonstrated that the gut microbial enterotype was altered in red meat diets, suggesting that high red meat consumption decreased *Clostridium* (phylum *Firmicutes*) and increased *Proteobacteria* including *Enterobacteriaceae,* with significant nitrate-reducing capacities [42]. Therefore, meat-derived substances such as nitrate and nitrite may affect the microbial composition and result in not only subsequent NOC formation but also microbial imbalance, which might be a more important intestinal environment for the development of cancer, which is particularly observed in patients with inflammatory bowel diseases (IBDs) (Fig. 1).

6. *N*-nitroso compound formation in inflammatory bowel diseases

An increased incidence of colorectal cancer has been observed in patients with IBDs such as ulcerative colitis. Intense exposure to nitrate in drinking water is associated with an increased risk of colon cancer in patients with IBD, possibly via bacterial and chemical NOC formation [25]. It provides evidence of carcinogenicity for exogenous nitrate and nitrite in these patients [23]. On the other hand, submucosal iNOS activity is increased in patients with IBD, and excessive NO is endogenously released in the vicinity of the mucosal epithelia. Because there is a steep radial oxygen gradient in the colon between the aerobic submucosa and the anaerobic luminal center, with partial oxygen pressure (pO_2) ranging from 100 to 0.1 mmHg [46], NO released from iNOS of submucosal inflammatory neutrophils is oxidized to nitrite and nitrate in the aerobic inner mucous layer of the colon [70]. It then serves as a suitable electron acceptor for nitrate and nitrite-reducing anaerobes to generate metabolic energy (nitrate respiration) [1].

Although intestinal microbiota, including most commensal obligate anaerobic microbiota, benefit the host by stimulating the development of the immune system, supplying nutrients (e.g., short chain fatty acids and vitamins), and providing niche protection in healthy patients, an excess of NO that is derived from endogenous production as well as dietary sources might cause a dysbiotic microbial community and increase the patient's susceptibility to cancer development. Contrary to gastric carcinogenesis, which seems to result from a single pathogen such as *Helicobacter pylori*, dysbiosis

in the colon shows a shift in microbiota from obligate anaerobes to respiratory nitrate or nitrite-reducing facultative anaerobes of *Proteobacteria* and *Firmicutes* phyla by edging out the competing microbes that depend on fermentation to generate energy for growth. This shift is particularly notable in the context of IBD, in which massive blooms of pathogenic nitrate-reducing *Salmonella* and *Escherichia coli* are observed in response to bowel inflammation [91]. This dysbiosis disrupts the luminal microenvironment and not only causes carcinogenic NOC formation but also contributes to the impaired NO signaling that is responsible for promoting further chronic inflammation and subsequent cancer development. A further detailed investigation concerning whether dysbiosis is the cause or result of NOC formation is required to determine the exact relationship between dysbiosis and colorectal cancer.

7. Conclusion

In conclusion, dietary nitrate and nitrite might have benefits and deficits in cancer biology, depending on which nitroso compound (*S*-nitroso or *N*-nitroso) is favorably formed in the GI tract.

Vegetables rich in nitrate are a major source of nitrosating precursors in the stomach via the enterosalivary pathway. The nitrosating process of salivary nitrite to beneficial *S*-nitrosation or carcinogenic *N*-nitrosation is prominently influenced by gastric acidity with more RS-NO and less RN-NO in decreased gastric pH. Additional dietary antioxidants facilitate more generation of NO and RS-NO, providing local and systemic physiological benefits. Thus, most dietary nitrate and nitrite are catalyzed and absorbed in the upper GI tract before they reach the colon. However, an increased gastric pH could make the stomach a stage for NOC formation.

The lower GI tract is another stage for NOC formation. Red meat is the main dietary source of iron and amino acids essential for human health. However, too much heme protein might become a possible transporter of nitrosating precursors to the colon and influence the colonal microbial flora, allowing more NOC formation in the colon. In addition, NOC formation in the colon is also increased by endogenous NO generation due to IBDs.

Basing on the recent evidence on the physiological aspect of dietary nitrate, I believe that dietary nitrate and gut environment might play important roles not only in physiological health maintenance but also in carcinogenic NOC formation and GI cancer development.

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References

[1] L. Albenberg, T.V. Esipova, C.P. Judge, K. Bittinger, J. Chen, A. Laughlin, S.R. Thom, Correlation between intraluminal oxygen gradient and radial partitioning of intestinal microbiota, Gastroenterology. 147 (2014) 1055-1063.

[2] J. Alexander, D. Benford, A. Cockburn, J.P. Cravedi, E. Dogliotti, A.D. Domenico, P. Grandjeam, Nitrate in vegetables scientific opinion of the panel on contaminants in the food chain, EFSA J. 689 (2008) 1-79.

[3] J.H. Amaral, M.F. Montenegro, L.C. Pinheiro, G.C. Ferreira, R.P. Barroso, A.J. Costa-Filho, J.E. Tanus-Santos, TEMPOL enhances the antihypertensive effects of sodium nitrite by mechanisms facilitating nitrite-derived gastric nitric oxide formation, Free Radic. Biol. Med. 65 (2013) 446-455.

[4] D.L. Archer, Evidence that ingested nitrate and nitrite are beneficial to health, J. Food Protect. 65 (2002) 872-875.

[5] H. Bartsch, H. Ohshima, B. Pignatelli, Inhibitors of endogenous nitrosation. Mechanism and implications in human cancer prevention, Mutat. Res. 202 (1988) 307-324.

[6] N.M. Bastide, F. Chenni, M. Audebert, R.L. Santarelli, S. Tache, N. Naud, M. Baradat, I. Jouanin, R. Surya, D.A. Hobbs, G.G. Kuhnle, I. Raymond-Letron, F. Gueraud, D.E. Corpet, F.H.F. Pierre, A central role for heme iron in colon carcinogenesis associated with red meat intake, Cancer Res. 75 (2015) 870-879.

[7] C. Bernstein, H. Holubec, A.K. Bhattacharyya, H. Nguyen, C.M. Payne, B. Zaitlin, H. Bernstein, Carcinogenecity of deoxycholate, a secondary bile acid, Arch. Toxicol. 85 (2011) 863-871.

[8] S.A. Bingham, B. Pignatelli, J.R.A. Pollock, A. Ellul, C. Malaveille, G. Gross, S. Runswick, J.H. Cummings, I.K.O. Neill, Does increased endogenous formation of N-nitroso compounds in the human colon explain the association between red meat and colon cancer? Carcinogenesis 17 (1996) 515-523.

[9] S.A. Bingham, R. Hughes, A.J. Cross, Effect of white meat versus red meat on endogenous *N*-nitrosation in the human colon and further evidence of a dose response, J. Nutr. 132 (2002) 3522S-3525S.

[10] R. Bonnett, A.A. Charalambides, R.A. Martin, K.D. Sales, B.W. Fitzsimmons, Reactions of nitrous acid and nitric oxide with porphyrins and haems. Nitrosylhaems as nitrosating agents, J. Chem. Soc. Chem. Commun. 21 (1975) 884-885.

[11] J.L. Bos, *Ras* oncogenes in human cancer: a review, Cancer Res. 49 (1989) 4682-4689.

[12] V. Bouvard, D. Loomis, K.Z. Guyton, Y. Grosse, F.E. Ghissassi, L. Benbrahim-Tallaa, N. Guha, H. Mattock, K. Straif, Carcinogenicity of consumption of red and processed meat, Lancet Oncol. 16 (2015) 1599-1600.

[13] K.E. Bradbury, P.N. Appleby, T.J. Key, Fruit, vegetable, and fiber intake in relation to cancer risk: findings from the European Prospective Investigation into Cancer and Nutrition (EPIC), Am. J. Clin. Nutr. 100 (2014) 394S-398S.

[14] N.A. Bryan, D.D. Alexander, J.R. Coughlin, A.L. Milkowaki, P. Boffetta, Ingested nitrate and

nitrite and stomach cancer risk: An updated review, Food Chem. Toxicol. 50 (2012) 3646-3665.

[15] J.V. Bussche, L.Y. Hemeryck, T. Van Hecke, G.G. Kuhnle, F. Pasmans, S.A. Moore, T. Van de Wiele, S.D. Smet, L. Vanhaecke, O^6-carboxymethylguanine DNA adduct formation and lipid peroxidation upon in vitro gastrointestinal digestion of haem-rich meat, Mol. Nutr. Food Res 58 (2014) 1883-1896.

[16] S. Calmels, H. Ohshima, P. Vincent, A.M. Gounot, H. Bartsch, Screening of microorganisms for nitrosation catalysis at pH 7 and kinetic studies on nitrosamine formation from secondary amines by E. coli strains, Carcinogenesis. 6 (1985) 911-915.

[17] S. Calmels, H. Ohshima, H. Rosenkranz, E. McCoy, H. Bartsch, Biochemical studies on the catalysis of nitrosation by bacteria, Carcinogenesis. 8 (1987) 1085-1088.

[18] S. Calmels, H. Ohshima, H. Bartsch, Nitrosamine formation by denitrifying and non-denitrifying bacteria: implication of nitrite reductase and nitrite reductase in nitrosation catalysis, J. Gen. Microbiol. 134 (1988) 221-226.

[19] S. Calmels, H. Ohshima, Y. Henry, H. Bartsch, Characterization of bacterial cytochrome cd_1-nitrite reductase as one enzyme responsible for catalysis of nitrosation of secondary amines, Carcinogenesis. 17 (1996) 533-536.

[20] J.L. Caulfield, S.P. Singh, J.S. Wishnok, W.M. Deen, S.R. Tannenbaum, Bicarbonate inhibits N-nitrosation in oxygenated nitric oxide solutions, J. Biol. Chem. 271 (1996) 25859-25863.

[21] N.L. Chan, P.H. Rogers, A. Arnone, Crystal structure of the S-nitroso form of liganded human hemoglobin, Biochemistry. 37 (1998) 16459-16464.

[22] A.J. Cross, J.R.A. Pollock, S.A. Bingham, Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat, Cancer Res. 63 (2003) 2358-2360.

[23] T.M.C.M. de Kok, L.G.J.B. Engels, E.J. Moonen, J.C.S. Kleinjans, Inflammatory bowel disease stimulates formation of carcinogenic N-nitroso compounds, Gut. 54 (2005) 731.

[24] C.T. DellaValle, Q. Xiao, G. Yang, X.O. Shu, B. Aschebrook-Kilfoy, W. Zheng, H.L. Li, B.T. Ji, N. Rothman, W.H. Chow, Y.T. Gao, M.H. Ward, Dietary nitrate and nitrite intake and risk of colorectal cancer in the Shanghai women's health study, Int. J. Cancer. 134 (2014) 2917-2926.

[25] A.J. De Roos, M.H. Ward, C.F. Lynch, K.P. Cantor, Nitrate in public water supplies and the risk of colon and rectum cancers, Epidemiology. 14 (2003) 640-649.

[26] A. Engemann, C. Focke, H.U. Humpf, Intestinal formation of N-nitroso compounds in the pig cecum model, J. Agric. Food Chem. 61 (2013) 998-1005.

[27] T.H. Florin, G. Neale, J.H. Cummings, The effect of dietary nitrate on nitrate and nitrite excretion in man, Br. J. Nutr. 64 (1990) 387-397.

[28] B. Gago, T. Nyström, C. Cavaleiro, B.S. Rocha, R.M. Barbosa, J. Laranjinha, J.O. Lundberg, The potent vasodilator ethyl nitrite is formed upon reaction of nitrite and ethanol under gastric conditions, Free Radic. Biol. Med. 45 (2008) 404-412.

[29] M. Greenblatt, S. Mirvish, B.T. So, Nitrosamine studies: introduction of lung adenomas by concurrent administration of sodium nitrite and secondary amines in Swiss mice, J. Natl. Cancer Inst.

46 (1971) 1029-1034.

[30] Y. Grosse, R. Baan, K. Straif, B. Secretan, F.E.I. Ghissassi, V. Cogliano, Carcinogenicity of nitrate, nitrite, and cyanobacterial peptide toxins, Lancet Oncol. 7 (2006) 628-629.

[31] F. Guarner, J.R. Malagelada, Gut flora in health and disease, Lancet. 360 (2003) 512-519.

[32] M. Habermeyer, A. Roth, S. Guth, P. Diel, K.H. Engel, B. Epe, P. Furst, V. Heinz, H.U. Humpf, H.G. Joost, D. Knorr, T. de Kok, S. Kulling, A. Lampen, D. Marko, G. Rechkemmer, I. Rietjens, R.H. Stadler, S. Vieths, R. Vogel, P. Steinberg, G. Eisenbrand, Nitrate and nitrite in the diet: How to assess their benefit and risk for human health, Mol. Nutr. Food Res. 59 (2015) 106-128.

[33] H.M. Hamer, D.M.A.E. Jonkers, A. Bast, S.A.L.W. Vanhoutvin, M.A.J.G. Fisher, A. Kodde, F.J. Troost, K. Venema, R.J.M. Brummer, Butyrate modulates oxidative stress in the colonic mucosa of healthy humans, Clin. Nutr. 28 (2009) 88-93.

[34] G.M. Hawksworth, M.J. Hill, Bacteria and the *N*-nitrosation of secondary amines, Br. J. Cancer. 25 (1971) 520-526.

[35] M.A. Helser, J.H. Hotchkiss, D.A. Roe, Influence of fruit and vegetable juices on the endogenous formation of *N*-nitrosoproline and *N*-nitrosothiazolidine-4-carboxylic acid in humans on controlled diets, Carcinogenesis. 13 (1992) 2277-2280.

[36] M.N. Hughes, Relationship between nitric oxide, nitroxyl ion, nitrosonium cation and peroxynitrite, Biochem. Biophys. Acta. 1411 (1999) 263-272.

[37] R. Hughes, I.R. Rowland, Metabolic activities of the gut microflora in relation to cancer, Microb. Ecol. Health Dis. Suppl. 2 (2000) 179-185.

[38] R. Hughes, E.A.M. Magee, S. Bingham, Protein degradation in the large intestine: relevance to colorectal cancer, Curr. Issues Intest. Microbiol. 1 (2000) 51-58.

[39] R. Hughes, A.J. Cross, J.R.A. Pollock, S. Bingham, Dose-dependent effect of meat on endogenous colonic *N*-nitrosation, Carcinogenesis. 22 (2001) 199-202.

[40] R. Hughes, J.R. Pollock, S. Bingham, Effect of vegetables, tea, and soy on endogenous *N*-nitrosation, fecal ammonia, and fecal water genotoxicity during a high red meat diet in humans, Nutr. Cancer. 42 (2002) 70-77.

[41] K. Iijima, E. Henry, A. Moriya, A. Wirz, A.W. Kelman, K.E.L. McColl, Dietary nitrate generates potentially mutagenic concentrations of nitric oxide at the gastroesophageal junction, Gastroenterology. 122 (2002) 1248-1257.

[42] N. IJssennagger, M. Derrien, G.M. Van Doorn, A. Rijnierse, B. Van den Bogert, M. Muller, J. Dekker, M. Kleerebezem, R. van der Meer, Dietary heme alters microbiota and mucosa of mouse colon without functional changes in host-microbe cross-talk, PLOS. One. 7 (2012) 1-10.

[43] A.M.C.P. Joosen, G.G.C. Kuhnle, S.M. Aspinall, T.M. Barrow, E. Lecommandeur, A. Azqueta, A.R. Collins, S.A. Bingham, Effect of processed and red meat on endogenous nitrosation and DNA damage, Carcinogenesis. 30 (2009) 1402-1407.

[44] F. Khanum, K.R. Anilakumar, K.R. Viswanathan, Anticarcinogenic properties of garlic: a review. Crit. Rev. Food Sci. Nutr. 44 (2004) 479-488.

[45] J. Kobayashi, K. Ohtake, H. Uchida, NO-rich diet for lifestyle-related diseases, Nutrients 7 (2015) 4911-4937.

[46] C.D. Koch, M.T. Gladwin, B.A. Freeman, J.O. Lundberg, E. Weinzberg, Enterosalivary nitrate metabolism and the microbiome: Intersection of microbial metabolism, nitric oxide and diet in cardiac and pulmonary vascular health, Free Radic. Biol. Med. In press, 2016. http://doi.org/10.1016/j.freeradbiomed.2016.12.015.

[47] G.G.C. Kuhnle, G.W. Story, T. Reda, A.R. Mani, K.P. Moore, J.C. Lunn, S.A. Bingham, Diet-induced endogenous formation of nitroso compounds in the GI tract, Free Radic. Biol. Med. 43 (2007) 1040-1047.

[48] N. Kunisaki, M. Hayashi, Formation of *N*-nitrosamines from secondary amines and nitrite by resting cells of Escherichia coli B, Appl. Environ. Microbiol. 37 (1979) 279-282.

[49] V.M. Lakshmi, M.L. Clapper, W.C. Chang, T.V. Zenser, Hemin potentiates nitric oxide-mediated nitrosation of 2-amino-3-methylimidazo[4,5-f]quinolone(IQ) to 2-nitrosoamino-3-methylimidazo [4,5-f]quinolone, Chem. Res. Toxicol. 18 (2005) 528-535.

[50] S.A. Leach, M. Thompson, M. Hill, Bacterially catalysed *N*-nitrosation reactions and their relative importance in the human stomach, Carcinogenesis. 8 (1987) 1907-1912.

[51] S.A. Leach, C.W. Mackerness, M.J. Hill, M.H. Thompson, Inhibition of bacterially mediated *N*-nitrosation by ascorbate: therapeutic and mechanistic considerations, IARC Sci. Publ. 105 (1991) 571-578.

[52] W.J. Lee, K. Hase, Gut microbiota-generated metabolites in animal health and disease, Nat. Chem. Biol. 10 (2014) 416-424.

[53] M.H. Lewin, N. Bailey, T. Bandalletova, R. Bowman, A.J. Cross, J. Pollock, D.E.G. Shuker, S.A. Bingham, Red meat enhances the colonic formation of the DNA adduct O^6-carboxymethyl guanine: implications for colorectal cancer risk, Cancer Res. 66 (2006) 1859-1865.

[54] W. Lijinsky, Introduction of tumours in rats by feeding nitrosatable amines together with sodium nitrite, Food Chem. Toxicol. 22 (1984) 715-720.

[55] Y.H. Loh, P. Jakszyn, R.N. Luben, A.A. Mulligan, P.N. Mitrou, K.T. Khaw, *N*-nitroso compounds and cancer incidence: the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study, Am. J. Clin. Nutr. 93 (2011) 1053-1061.

[56] W.M. Loke, J.M. Hodgson, J.M. Proudfoot, A.J. McKinley, I.B. Puddey, K.D. Croft, Pure dietary flavonoids quercetin and (-)-epicatechin augment nitric oxide products and reduce endothelin-1 acutely in healthy men, Am. J. Clin. Nutr. 88 (2008) 1018-1025.

[57] J.C. Lunn, G. Kuhnle, V. Mai, C. Frankenfeld, D.E.G. Shuker, R.C. Glen, J.M. Goodman, J.R.A. Pollock, S.A. Bingham, The effect of haem in red and processed meat on the endogenous formation of *N*-nitroso compounds in the upper GI tract, Carcinogenesis. 28 (2007) 685-690.

[58] R.C. Massey, P.E. Key, A.K. Mallett, I.R. Rowland, An investigation of the endogenous formation of apparent total *N*-nitroso compounds in conventional microflora and germ-free rats, Food Chem. Toxicol. 26 (1988) 595-600.

[59] S.S. Mirvish, M.E. Davis, M.P. Lisowyj, N.W. Gaikwad, Effect of feeding nitrite, ascorbate, hemin, and omeprazole on excretion of fecal total apparent *N*-nitroso compounds in mice, Chem. Res. Toxicol. 21 (2008) 2344-2351.

[60] A. Nowak, S. Kuberski, Z. Libudzisz, Probiotic lactic acid bacteria detoxify *N*-nitrosodimethylamine, Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 31 (2014) 1678-1687.

[61] C.M. O'Donnell, C. Edwards, J. Ware, Nitrosamine formation by clinical isolates of enteric bacteria, FEMS Microbiol. Lett. 51 (1988) 193-197.

[62] C.M. O'Donnell, C. Edwards, Nitrosating activity in Escherichia coli, FEMS Microbiol. Lett. 74 (1992) 95-98.

[63] K. Ohtake, N. Ehara, H. Chiba, G. Nakano, K. Sonoda, J. Ito, H. Uchida, J. Kobayashi, Dietary nitrite reverses features of postmenopausal metabolic syndrome induced by high-fat diet and ovariectomy in mice, Am. J. Physiol. Endo. Metab. 312 (2017) E300-E308.

[64] A.S. Pannala, A.R. Mani, J.P.E. Spencer, V. Skinner, K.R. Bruckdorfer, K.P. Moore, C.A. Rice-Evans, The effect of dietary nitrate on salivary, plasma, and urinary nitrate metabolism in humans, Free Radic. Biol. Med. 34 (2003) 576-584.

[65] F.H.F. Pierre, O.C.B. Martin, R.L. Santarelli, S. Tache, N. Naud, F. Gueraud, M. Audebert, J. Dupuy, N. Meunier, D. Attaix, J.L. Vendeuvre, S.S. Mirvish, G.C.G. Kuhnle, N. Cano, D.E. Corpet, Calcium and α-tocopherol suppress cured-meat promotion of chemically induced colon carcinogenesis in rats and reduce associated biomarkers in human volunteers, Am. J. Clin. Nutr. 98 (2013) 1255-1256.

[66] B. Pignatelli, C. Malaveille, A. Rogatko, A. Hautefeuille, P. Thuillier, N. Munoz, B. Moulinier, F. Berger, H. De Montclos, R. Lambert, Mutagens, *N*-nitroso compounds and their precursors in gastric juice from patients with and without precancerous lesions of the stomach, Eur. J. Cancer. 29A (1993) 2031-2039.

[67] L.C. Pinheiro, J.H. Amaral, G.C. Ferreira, R.L. Portella, C.S. Ceron, M.F. Montenegro, J.E. Tanus-Santos, Gastric *S*-nitrosothiol formation drives the antihypertensive effects of oral sodium nitrite and nitrate in a rat model of renovascular hypertension, Free Radic. Biol. Med. 87 (2015) 252-262.

[68] D. Ralt, J.S. Wishnok, S.R. Tannenbaum, Bacterial catalysis of nitrosation: involvement of the nar operon of Escherichia coli, J. Bacteriol. 170 (1988) 359-364.

[69] P.I. Reed, P.L. Smith, K. Haines, House FR, Walter CL, Effect of cimetidine on gastric juice *N*-nitrosamine concentration, Lancet. 318 (1981) 553-556.

[70] W.E.W. Roediger, M.J. Lawson, B.C. Radcliffe, Nitrite from inflammatory cells. A cancer risk factor in ulcerative colitis, Dis. Colon Rectum. 33 (1990) 1034-1036.

[71] I.R. Rowland, T. Granli, O.C. Bøckman, P.E. Key, R.C. Massey, Endogenous *N*-nitrosation in man assessed by measurement of apparent total N-nitroso compounds in faeces, Carcinogenesis. 12 (1991) 1395–1401.

[72] W.S. Ruddell, E.S. Bone, M.J. Hill, L.M. Blendis, C.L. Walters, Gastric-juice nitrite. A risk factor for cancer in the hypochlorhydric stomach? Lancet. 13 (1976) 1037-1039.

[73] C.J. Rumney, I.R. Rowland, T.M. Coutts, K. Randearth, R. Reddy, A.B. Shah, A. Ellul, I.K. O'Neill, Effects of risk-associated human dietary macrocomponents on processes related to carcinogenesis in human-flora-associated HFA) rats, Carcinogenesis. 14 (1993) 79-84.

[74] W.R. Russell, S.W. Gratz, S.H. Duncan, G. Holtrop, J. Ince, L. Scobbie, G. Duncan, A.M. Johnstone, G.E. Lobley, R.J. wallance, G.G. Duthie, H.J. Flint, High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health, Am. J. Clin. Nutr. 93 (2011) 1062-1072.

[75] R.L. Saul, S.H. Kabir, Z. Cohen, W.R. Bruce, M.C. Archer, Reevaluation of nitrate and nitrite levels in the human intestine, Cancer Res. 41 (1981) 2280-2283.

[76] T. Sawa, T. Akaike, K. Kida, Y. Fukushima, K. Takagi, H. Maeda, Lipid peroxyl radicals from oxidized oils and heme-iron: implication of a high-fat diet in colon carcinogenesis, Cancer Epidem. Biomar. 7 (1998) 1007-1012.

[77] N.R. Shenoy, A.S. Choughuley, Inhibitory effect of diet related sulphydryl compounds on the formation of carcinogenic nitrosamines, Cancer Lett. 65 (1992) 227-232.

[78] K.R. Silvester, S.A. Bingham, J.R.A. Pollock, J.H. Cummings, I.K. O'Neill, Effect of meat and resistant starch on fecal excretion of apparent *N*-nitroso compounds and ammonia from the human large bowel, Nutr. Cancer. 29 (1997) 13-23.

[79] G.M. Sobala, C.J. Schorah, M. Sanderson, M.F. Dixon, D.S. Tompkins, P. Godwin, A.T. Axon, Ascorbic acid in the human stomach, Gastroenterology. 97 (1989) 257-363.

[80] T. Sobko, C. Reinders, E. Norin, T. Midtvedt, L.E. Gustafsson, J.O. Lundberg, Gastrointestinal nitric oxide generation in germ-free and conventional rats, Am. J. Physiol. Gastrointest. Liver Physiol. 287 (2004) G993-G997.

[81] T. Sobko, C. Reinders, E.Å. Jansson, E. Norin, T. Midtvedt, J.O. Lundberg, Gastrointestinal bacteria generate nitric oxide from nitrate and nitrite, Nitric Oxide 13 (2005) 272-278.

[82] K. Sonoda, K. Ohtake, H. Uchida, J. Ito, M. Uchida, H. Natsume, H. Tamada, J. Kobayashi, Dietary nitrite supplementation attenuates cardiac remodeling in l-NAME-induced hypertensive rats, Nitric Oxide 67 (2017) 1-9.

[83] G.J.A. Speijers, P.A. van den Brandt, Joint FAO & WHO Expert Committee on Food Additives, Nitrate and potential endogenous formation of N-nitroso compounds, in: Toxicological Evaluation of Certain Food Additives and Naturally Occurring Toxicants, prepared by the 59th Meeting of the JECFA, WHO Food Additives series 50 (2003).

[84] R.W. Stockbrugger, P.B. Cotton, N. Eugenides, B.A. Bartholomew, M.J. Hill, C.L. Walters, Intragastric nitrites, nitrosamines, and bacterial overgrowth during cimetidine treatment, Gut. 23 (1982) 1048-1054.

[85] K. Suzuki, T. Mitsuoka, *N*-nitrosamine formation by intestinal bacteria, IARC. Sci. Publ. 57 (1984) 275-281.

[86] S.R. Tannenbaum, J.S. Wishnok, C.D. Leaf, Inhibition of nitrosamine formation by ascorbic acid, Am. J. Clin. Nutr. 53 (1991) 247S-250S.

[87] K. Vipperla, S.J. O'Keefe, Intestinal microbes, diet, and colorectal cancer, Curr. Colorectal Cancer Rep. 9 (2013) 95-105.

[88] A.J. Watson, P.D. Collins, Colon cancer: a civilization disorder, Dig. Dis. 29 (2011) 222-228.

[89] J.H. Weisburger, B.S. Reddy, T. Narisawa, E.L. Wynder, Germ-free status and colon tumor induction by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, Proc. Soc. Exp. Biol. Med. 148 (1975) 1119-1121.

[90] E. Weitzberg, J.O. Lundberg, Novel aspects of dietary nitrate and human health, Annu. Rev. Nutr. 33 (2013) 129-159.

[91] S.E. Winter, M.G. Winter, M.N. Xavier, P. Thiennimitr, V. Poon, A.M. Keestra, R.C. Laughlin, G. Gomez, J. Wu, S.D. Lawhon, I.E. Popova, S.J. Parikh, G. Adams, R.M. Tsolis, V.J. Stewart, A.J. Baumler, Host-derived nitrate boosts growth of *E. coli* in the inflamed gut, Science. 339 (2013) 708-711.

[92] Y.N. Wu, H.Z. Wang, J.S. Li, C. Han, The inhibitory effect of Chinese tea and its polyphenols on in vitro and in vivo *N*-nitrosation, Biomed. Environ. Sci. 6 (1993) 237-258.

[93] Y. Zhu, X. Lin, H. Li, Y. Shi, F. Zhao, X. Xu, C. Li, G. Zhou, Intake of meat proteins substantially increased the relative abundance of genus Lactobacillus in rat feces, PLOS. One. 11 (2016) e0152678.

Figure Legends

Figure 1

NOCs are mainly formed in stomach and lower intestine via bacterial, chemical and inflammatory pathways. 1) Bacterial NOC formation: Unless dietary nitrate/nitrite are filtered out by gastric acidity and antioxidants, they can reach the lower intestine as precursors of nitrosating agents. Intake of nitrate in drinking water alone particularly from private wells contaminated by nitrogen (use of nitrogen fertilizers) might be not only precursors of nitrosating agents but also harmful to the infants fed with artificial milk equipped with this water (methemoglobinemia). 2) Chemical NOC formation: Acid-catalyzed heme-NO provides nitrosator for NOC formation in the lower intestine. 3) Inflammatory NOC formation: Inflammatory bowel diseases endogenously provide nitrite and subsequently cause dysbiosis and NOC formation.

NO: nitric oxide, RN-NO (NOC): *N*-nitroso compound, RS-NO: *S*-nitroso compound, iNOS: inducible NO synthase

Table 1　　　Diets, nutrients and medicines affecting NOC (ATNC) formation in the GI tract

Diets, nutrients, drugs	Fecal (gastric) NOC levels	Species	Mechanisms	ref.
nitrate in drinking water (300mg nitrate/day)	increases NOC levels	human	nitrosating donor	71
dietary preformed NOC (hot dog, cured meat)	increases NOC levels	human	intake of NOC	55,59
red meat	increases NOC levels	human	heme-mediated nitrosation	Table 2
soy	decreases ATNC enhanced with red meat diet	human	reduced intestinal transit time	40
ascorbic acid	decreases ATNC enhanced with $NaNO_2$ in drinking water	human	antioxidant, inhibitory effect on nitrosation	59
green tea or black tea drinking after meal	decreases NOC enhanced with meal	human	possibly related to polyphenol content in tea	92
vegetables	decreases ATNC enhanced by 15 days' red meat diet	human	antioxidant, inhibitory effect on nitrosation	22
cimetidine (H_2 receptor antagonist)	increases NOC levels (in gastric juice)	human	increase in nitrite and intragastric bacterial overgrowth	69
omeprazole (proton pump inhibitor)	decreases ATNC enhanced with $NaNO_2$ in drinking water	human	decrease in acid-catalyzed nitrosation	59
high protein low carbohydrate and fiber diet(weight- loss diet)	increases NOC levels	human	increase in protein fermentation and decrease in carbohydrate fermentation	74
calcium carbonate	decreases ATNC enhanced with cured meat	human, rat	bind to dietary heme iron and suppress its toxicity	65
α-tocopherol		rat	antioxidant, inhibitory effect on nitrosation	

NOC: *N*-nitrosocompound,　ATNC: apparent total *N*-nitrosocompound, GI: gastrointestinal

Table 2 Human studies showing the effects of meat diet on fecal levels of NOC (ATNC) and nitrite

Intake	Protein/day	Fecal *N*-nitroso-compound (mean)		Fecal nitrite (nitrate)	ref.
free-choice western style diet				5-19 μM/kg (0-14 μM/kg)	75
normal free-choice diet		40-590 μg/kg			
diet low in nitrate		40-143 (82) μg/kg	ATNC		71
nitrate 300mg/day in water		73-714 (307) μg/kg			
free diet				59.0±39.0 mg/day	
low meat diet	60g	40.0±7.0 μg/day	ATNC	54.0±16.0 mg/day	8
high red meat diet	600g	113.0±25.0 μg/day		80.0±7.0 mg/day	
high white meat diet	600g			46.0±7.0 mg/day	
low meat diet	60g	35 μg/day	ATNC		78
high red meat diet	600g	114 μg/day			
	0g	444.0±59.4 μg/kg		180.0±40.0 μg/kg	
red meat diet	60g	374.0±61.4 μg/kg	ATNC	110.0±30.0 μg/kg	39
	240g	1516.1±414.3 μg/kg		500.0±200.0 μg/kg	
	420g	1980.8±567.8 μg/kg		420.0±130.0 μg/kg	
white meat diet	420-600g	759.6±528.0 μg/kg			
red meat (low) diet	60g	572.0±349.0 μg/kg	ATNC		9
red meat (high) diet	420-600g	2104.0±1524.0 μg/kg			
vegetable diet		349.6±46.8 μg/kg		300.7±69.6 μg/kg	
red meat (low) diet	60g	301.6±48.3 μg/kg	ATNC	221.3±37.0 μg/kg	22
red meat (high) diet	420g	1279.5±238.9 μg/kg		578.0±104.1μg/kg	
vegetable diet (+30g fiber)		193±43 μg/day			
red meat diet (+13g fiber)	420g	667±141 μg/day	ATNC		53
red meat diet	420g	7338±1973 μg/day			
red meat diet (+30g fiber)	420g	3871±1093 μg/kg			
vegetable diet		0.2±0.1nM/g	RS-NO	1.6±1 nM/mg (25±21 nM/mg)	
		0.2±0.03 nM/g	Fe-NO		47
red meat diet	360g	54.7±36.9 nM/g	RS-NO	14.0±2 nM/mg (24±3.9 nM/mg)	
		91.6±0.03nM/g	Fe-NO		
vegetable diet		2.6±0.3 nM/g	NOC		
(negligible heme)		0.2±0.1 nM/g	RS-NO		
		2.0±0.3 nM/g	Fe-NO		
processed meat diet	420g	181±20 nM/g	NOC		
(heme=86mM/day)		33±4 nM/g	RS-NO		
		95±9 nM/g	Fe-NO		43
vegetable diet		3.5±0.7 nM/g	NOC		
(negligible heme)		0.4±0.1 nM/g	RS-NO		
		1.8±0.3 nM/g	Fe-NO		
red meat diet	420g	177±26 nM/g	NOC		
(heme=110mM/day)		19±4 nM/g	RS-NO		
		140±20 nM/g	Fe-NO		

NOC: *N*-nitrosocompound,   ATNC: apparent total *N*-nitrosocompound,   RS-NO: *S*-nitrosothiol, Fe-NO iron nitrosyl

Table 3 *in vitro* **NOC formation by intestinal bacteria**

bacteria	study conditions	nitrosating agent	nitrosatable substrate	ref.
E.coli	*in vitro*, pH=6.5, aerobic	nitrate/nitrite	diphenylamine, pyrrolidine dimethylamine, diethylamine, *N*-methyl aniline, piperidine,	
Enterococci	*in vitro*, pH=6.5, aerobic			34
Clostridia, bacteroides, Bifidobacteria	*in vitro*, pH=6.5, anaerobic	nitrite	diphenylamine	
E.coli B	*in vitro*, pH=8	nitrite	dimethylamine piperidine	48
intragastric aerobic bacteria (mainly nitrate reducing bacteria)	*in vivo*, pH=5.34 after cimetidine treatment	nitrate/nitrite in gastric juice in the fasting state		84
E.coli A10 strain, *Proteus morganii*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*	*in vitro*, pH=7.5, aerobic	nitrite	morpholine, dimethylamine, diethylamine, dibutylamine, diisobutylamine, piperidine, pyrrolidine	85
Peptococcus asaccharolyticus	*in vitro*, pH=7.5, anaerobic			
E.coli A10 strain	*in vitro*, pH=7.25	nitrite	morpholine	16
E.coli A10 strain	*in vitro*, inhibited by cysteine or tungsten	nitrate/nitrite	morpholine	17
E.coli, Proteus morganii	*in vitro*, anaerobic neutral pH	nitrate but not nitrite	morpholine	18
Pseudomonas aeruginosa	*in vitro*, anaerobic neutral pH	nitrate/nitrite		
bacteria isolated from hypoacidic stomach	*in vitro*, some stimulated, some inhibited	nitrite	morpholine	61
E.coli	*in vitro*, anaerobic	nitrate/nitrite	2,3-diaminonaphthalene	68
Pseudomonas aeruginosa	*in vitro*, neutral pH, inhibited by ascorbate	nitrite	morpholine	51
E coli (NADH dependent nitrite reductase)	*in vitro*, aerobic	nitrite	morpholine	62
Pseudomonas aeruginosa, Neisseria mucosae (cytochrome cd_1-nitrite reductase) *E coli, Proteus morganii*	*in vitro*, neutral pH	nitrite	morpholine	19
Lactobacillus strains	*in vitro*, inhibited by lactic acid bacteria	nitrite	dimethylamine	60

NOC: *N*-nitrosocompound, NADH: nicotinamide adenine dinucleotide

Figure 1

Proposed schema showing the metabolism of dietary nitrate/nitrite and NOC formation in the gastrointestinal tract

oral bacteria

entero-salivary pathway

Nitrate

stomach

urine

Nitrite (nitrosating agent)

high pH, bacteria

low pH, antioxidants

(with ethanol)

RN-NO (**NOC**)

RS-NO NO ethyl nitrite

red meat (heme)

Nitrate/Nitrite

heme-NO

small intestine

Chemical formation

microbiota

NOC

iNOS

Bacterial formation

Inflammatory formation

large intestine