Haem, not Protein or Inorganic Iron, Is Responsible for Endogenous Intestinal N-Nitrosation Arising from Red Meat

Amanda Jane Cross, Jim R. A. Pollock, and Sheila Anne Bingham

Abstract

Many N-nitroso compounds (NOC) are carcinogens. In this controlled study of 21 healthy male volunteers, levels of NOC on a high (420 grams) red meat diet were significantly greater ($P = 0.001$) than on a low (60 grams) meat diet but not significantly greater when an equivalent amount of vegetable protein was fed. An 8-mg supplement of haem iron also increased fecal NOC ($P = 0.006$) compared with the low meat diet, but 35-mg ferrous iron had no effect. Endogenous N-nitrosation, arising from ingestion of haem but not inorganic iron or protein, may account for the increased risk associated with red meat consumption in colorectal cancer.

Introduction

Red and processed meat intake is associated with increased risk of colorectal cancer (1). Our previous studies in humans have established that red but not white meat stimulates endogenous intestinal N-nitrosation and that there is a dose response (2–4). This could be important for carcinogenesis in the large bowel because many classes of NOCs (2) have been identified, including nitrosamines, nitrosamides, and nitrosoguanidines, most of which are known carcinogens. After eating meat, the large intestine is rich in nitrogenous residues and nitrosating agents from protein metabolism and bacterial dissimilatory nitrate metabolism. In the present study, we show that it is haem iron, not protein residues or inorganic iron, that stimulates endogenous NOC production.

Materials and Methods

Two studies were carried out with volunteers living in a metabolic suite where all food and drink was provided and all specimens collected. The Cambridge Local Research Ethics Committee gave permission for the studies, and each volunteer signed a consent form after receiving a detailed explanation of the study protocol and aims. All volunteers were given a medical and completed a health and lifestyle questionnaire; subjects with a history of gastrointestinal disease or recent antibiotic use were excluded. Only foods and drinks provided by diet technicians, and weighed to the nearest gram from a diagnostic kit 171; Sigma, Poole, United Kingdom). The recoveries of fecal markers (97.9 and 97.7% for Protocols 1 and 2, respectively) were regarded as significant. From repeat analyses on subjects on high (420 grams) meat diets, the within-person SD was 56 ± 9.2 μg/day, and setting $α = 0.05$ and $β = 0.2$, the study had sufficient power to detect 65- and 75-μg differences in ATNC between study periods with 12 and 9 subjects, respectively. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement." The abbreviation used: NOC, N-nitroso compound; NO, nitric oxide; ATNC, apparent total N-nitroso compounds.
HAEM IS RESPONSIBLE FOR ENDOGENOUS N-NITROSATION

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

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

metabolic suite where diet could be carefully controlled (2–4, 7, 8). The direction of an increase with increasing red meat is consistent in nearly all individuals. Furthermore, there is a dose response, which occurs at normal levels of 120–240– and 420-gram red meat/day (2, 3). At the higher levels of red meat consumption, concentrations of ATNC are as the same order as the concentration of tobacco-specific NOC in cigarette smoke (4). We have shown previously that fermentable carbohydrate does not change faecal ATNC output (4, 7, 8) and that it is red, not white, meat which is responsible for the effect (2). It has been established that ATNC levels arise endogenously, because high red meat diets containing 600-gram meat per day provide only 13 μg of preformed ATNC per day (7).

We postulated that an increase in meat consumption would increase the amount of nitrogen residue reaching the large intestine, so that the substrates for nitrosating agents from protein metabolism and bacterial dissimilatory nitrate metabolism, and hence NOC levels, would increase (9). Fecal ammonia, also implicated in carcinogenesis, increased in this study in response to increased meat, as expected (9). To determine whether the reason for the increase in endogenous N-nitrosation arising from increased meat consumption was attributable to protein, we fed 143–150 grams of protein from mainly red meat or vegetarian sources in Protocol 1. However, endogenous NOC production increased when subjects were on the red meat diet, but the protein from vegetarian sources had no effect.

Our previous work was based on the supposition that N-nitrosation was brought about by bacteria colonizing the large intestine, because a study with germ-free rats showed that a normal microbial flora was required for endogenous N-nitrosation to occur (10). A number of

Table 1 Mean (±SE) faecal ATNC (μg/kg or μg/day) as NNO and nitrite for the two dietary protocols

<table>
<thead>
<tr>
<th>Protocol 1</th>
<th>Low red meat</th>
<th>High red meat</th>
<th>Vegetarian</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATNC (μg/kg)</td>
<td>301.6 ± 48.3</td>
<td>1279.5 ± 238.9</td>
<td>349.6 ± 46.8</td>
</tr>
<tr>
<td>ATNC (μg/day)</td>
<td>42.1 ± 5.3</td>
<td>190.1 ± 21.6</td>
<td>63.3 ± 5.3</td>
</tr>
<tr>
<td>Nitrite (μg/kg)</td>
<td>221.3 ± 37.0</td>
<td>578.0 ± 104.1</td>
<td>300.7 ± 69.6</td>
</tr>
<tr>
<td>Nitrite (μg/day)</td>
<td>39.7 ± 8.5</td>
<td>90.1 ± 15.0</td>
<td>70.0 ± 24.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protocol 2</th>
<th>Low red meat</th>
<th>Haem supplement</th>
<th>Inorganic iron supplement</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATNC (μg/kg)</td>
<td>766.4 ± 232.6</td>
<td>1438.0 ± 344.8</td>
<td>852.3 ± 392.9</td>
</tr>
<tr>
<td>ATNC (μg/day)</td>
<td>77.5 ± 9.0</td>
<td>156.8 ± 22.7</td>
<td>60.7 ± 9.5</td>
</tr>
<tr>
<td>Nitrite (μg/kg)</td>
<td>267.6 ± 31.3</td>
<td>712.4 ± 161.9</td>
<td>388.8 ± 116.2</td>
</tr>
<tr>
<td>Nitrite (μg/day)</td>
<td>33.5 ± 4.2</td>
<td>94.9 ± 29.7</td>
<td>43.4 ± 19.5</td>
</tr>
</tbody>
</table>

* P = 0.001 low versus high red meat diet.
* P < 0.001 meat versus meat diet.
* P = 0.006 low meat versus haem diet.
* P = 0.004 inorganic iron versus haem diet.
* P < 0.0001 low versus high red meat diet.
* P = 0.004 low meat versus haem diet.
* P = 0.001 inorganic iron versus haem diet.
* P = 0.007 low versus high red meat diet.

The table shows that differences in fecal nitrite between the low and high red meat diet in Protocol 1 were significant, but there were no other differences between diets. There were no significant differences in fecal weight or Mean Transit Time between study periods, apart from an increase in fecal weight from 165.4 ± 22.8 to 211.4 ± 27.2 grams/day (P = 0.018) between the low meat and vegetarian diets in Protocol 1. Fecal ammonia was higher on the high protein red meat and vegetarian diets than on the low protein low red meat diet; 32.4 ± 4.2 and 26.3 ± 4.4 compared with 20.3 ± 3 mmol/liter (P = 0.007, 0.062), and 5.3 ± 0.7 and 5 ± 1 compared with 2.9 ± 0.4 mmol/liter (P = 0.011, 0.015), respectively. However, there were no differences in fecal ammonia between the high protein diets of Protocol 1 or the low iron and iron-supplemented diets of Protocol 2.

Taking account of five previous studies from our laboratory, the influence of red meat on fecal ATNC excretion has now been shown in >60 healthy male volunteers, all of whom were studied in a study with germ-free rats showed that a normal microbial flora was brought about by bacteria colonizing the large intestine, because a study with germ-free rats showed that a normal microbial flora was required for endogenous N-nitrosation to occur (10). A number of

gen (r = 0.986 and 0.709 for Protocols 1 and 2, respectively), from samples collected on days 10, 13, and 15, suggest a high degree of dietary compliance.

Three volunteers did not wish to take part in the full protocol, so these individuals were randomized to the low meat and low meat supplemented with haem dietary periods. Therefore, results for inorganic iron are only available for 6 subjects.

Results and Discussion

Table 1 shows that in Protocol 1, fecal ATNC concentration was significantly greater on the high meat diet compared with the low meat diet (P = 0.001) but that the difference in ATNC concentration between the low meat and vegetarian diet was not significant (P = 0.2). Fig. 1 shows that there was individual variation in the extent of response to red meat but that all individuals had increased fecal ATNC levels on the high red meat diet. The low individual extent of response to red meat but that all individuals had increased fecal ATNC levels on the high haem diet, but all individuals had low levels of fecal ATNC on the inorganic iron diet. The individual variation in extent of response to the high haem diet is also apparent in Fig. 2.

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facultative and anaerobic bacteria from healthy humans, including those from feces, are able to catalyze the formation of NOCs at neutral pH via nitrate reductase (11, 12). The activity of this enzyme has been positively correlated with nitrosating ability (13), shown to vary $\approx$8-fold among individuals (14), and could thus explain individual variability in fecal ATNC levels. In this study, ATNC levels in some people were increased by as much as seven times over baseline values, whereas other levels only increased by $\sim$1.5 times (Figs. 1 and 2).

Red meat also contains iron, which is an integral part of bacterial nitrate reductase and could also explain the effect of red meat. Rats harboring a human fecal flora in the intestine and fed human diets showed a 3-fold increase in fecal nitrate reductase activity with a 3-fold increase in meat consumption (15). However, in Protocol 2, supplements of either haem iron or inorganic ferrous iron showed that only haem iron increased endogenous N-nitrosation. N-nitrosohaemoglobin and N-nitrosomyoglobin can be formed from the reaction of nitrite with hemoglobin and myoglobin (16). NO has also been shown to react directly with hemoglobin and myoglobin to produce NOCs (17). More specifically, the reaction of a haem containing mutant cytochrome-c-peroxidase with peroxide gave a product capable of oxidizing N-hydroxyguanidine or N-o-hydroxyarginine, resulting in the NOC N-nitrosoarginine (18). The finding that haem has an independent effect suggests that chemical catalysis, in addition to bacterial N-nitrosation, is responsible for the dose-dependent effect of red meat on increasing endogenous intestinal N-nitrosation. Should the NOCs formed endogenously in the intestine as a result of haem consumption be shown to be mutagenic or carcinogenic, this might explain the association between red meat consumption and large bowel cancer risk.

Acknowledgments

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References


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