Diet Composition Is Associated with Endogenous Formation of N-Nitroso Compounds in Obese Men1–3

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Abstract

Endogenous formation of carcinogenic N-nitroso compounds (NOC) occurs in the human gut. Red meat is considered the most important dietary component linked to NOC formation, although nitrate and vitamin C (VitC) also contribute. We previously showed that high-protein weight-loss diets increased fecal NOC and this was enhanced by simultaneous carbohydrate restriction. Although previous studies have focused on the effect of either 1 or 2 dietary components on endogenous NOC formation, no study to date has investigated the combined contribution of various dietary components. The current study therefore assessed the joint impact of several known dietary contributors to the endogenous formation of NOC in obese men. It also aimed to identify further novel contributors and investigate their role in explaining shifts in endogenous formation of NOC. Three dietary trials were conducted in obese men consuming body weight maintenance or weight-loss diets, with NOC measured in fecal samples. Consumption of meat-based weight-loss diets increased (P < 0.001) fecal NOC. Red meat intake was positively correlated with the fecal log NOC concentration (r = 0.60; P < 0.001). Dietary carbohydrate and sugar were negatively correlated with the fecal log NOC concentration (r = −0.66 for both; P < 0.001). Multiple regression analysis identified several dietary components that drive endogenous NOC formation, namely, red meat, nitrate, VitC, total energy, and nonstarch polysaccharides. We present a regression model that predicts endogenous NOC formation in obese men based on their dietary intakes. This model could improve the estimation of endogenous NOC formation, currently used in epidemiological studies into diet and cancer. J. Nutr. 142: 1652–1658, 2012.

Introduction

It is widely accepted that diet plays a crucial role in the development of gastrointestinal cancers. Following decades of epidemiological studies, the World Cancer Research Fund (WCRF)6 has listed red and processed meat as factors that increase the risk of developing colorectal cancer in humans (1,2). At the same time, red meat consumption remains high in many modern societies, with mean total red meat intake for UK men at 90.5 g/d in 2000-2001 and now exceeding 100 g/d (3). This is greater than the WCRF recommendation of no more than 500 g/wk red meat (1).

To explain this link between red meat consumption and risk of colorectal cancer, experimental studies have investigated the possible mechanisms involved. Notably, heme iron present in red meat in high concentrations has been suggested as a strong contributor to risk. Heme iron might directly act on the intestinal epithelium, increasing oxidative damage to DNA (4), inducing hyperproliferation (5), and increasing the risk of esophageal, gastric, and colon cancers in human (6,7). Furthermore, heme iron is thought to increase the endogenous formation of N-nitroso compounds (NOC) (8), some of which have been classified as probable human carcinogens by the International Agency for the Research of Cancer (9). NOC are detectable in food, especially in processed meat products, including bacon, ham, and sausages. NOC concentrations in human stool exceed dietary concentrations by at least 10-fold (10), suggesting endogenous formation in the human gut (11). Several dietary components have been suggested to contribute to endogenous formation of NOC, among which meat intake has been best described. A series of studies conducted by Bingham et al. (8,12–14) demonstrated the importance of meat intake, especially red...
meat intake and dietary heme, on the formation of NOC in the human gut. The authors also found that the addition of dietary fiber to high red meat diets did not counteract the increase in endogenous formation of NOC in men consuming large amounts of meat (10,13).

Another important dietary component driving endogenous nitrosation reactions is nitrate. Dietary intake of nitrate mainly derives from drinking water and vegetable sources (15). Oxidation of nitrate to nitrite might be performed by bacteria in the oral cavity, delivering free nitrite into the stomach for acid-catalyzed N-nitrosation to occur. Vitamin C (VitC) has been shown to counteract NOC formation from nitrate and amine precursors in gastric simulations in vitro and in humans (16–18). In addition to potential carcinogenic activity in the upper gut, NOC formed in the stomach are also considered to act as nitro-gen donors in the lower gut and contribute to NOC formation in the colon (19).

Published dietary intervention studies have identified specific dietary components that influence endogenous NOC formation, namely red meat and protein, carbohydrate, and dietary fiber, and also nitrate and VitC (8,10,18,20). To date, however, no studies to our knowledge have investigated the combined contributions of these dietary components on endogenous NOC formation in humans. Diets high in protein and meat and low in carbohydrate and fiber are commonly consumed to aid weight loss but may put overweight individuals following these diets at potential risk. The current study was therefore performed in obese men consuming various weight-loss diets. The design included 3 controlled dietary intervention trials, which allowed a detailed analysis of dietary intakes and changes in energy intakes for each volunteer. The work aimed to assess the individual and combined contributions of several known dietary intakes to endogenous NOC formation and to identify further novel contributors under conditions of reduced energy intake in obese men.

**Participants and Methods**

**Participant characteristics**

Obese but otherwise healthy men with a BMI >27 were recruited into 3 separate dietary intervention studies (different participants per study). The basal metabolic rate (BMR) was measured by indirect calorimetry as previously described (21) at the beginning and end of each dietary intervention period under standardized conditions after an overnight fast. The baseline characteristics of participants can be found in Supplemental Table 1. The participants were weight stable (<2 kg change in the past 3 mo) with no history of gastrointestinal problems. This was confirmed by a medical examination and by contacting participants’ general practitioners for medical history and recent medication status. No antibiotics or drugs known to influence the fecal microbiota were taken immediately prior to or during the course of the study. Ethical approval was granted by the North of Scotland Research Ethics Committee and all participants provided informed signed consent.

**Dietary interventions**

In each trial, all food was prepared and weighed by the kitchen research staff at the Human Nutrition Unit, with any leftovers weighed to the nearest gram. Participants completed food diaries, recording the time of each meal, with details of all food and drinks consumed. The detailed menus provided in each trial are summarized in Supplemental Table 2.

**Trial 1.** On entry, the participants (n = 16) were provided with a weight maintenance diet (MTD1; fed at 1.5 × BMR, containing 13% of total energy as protein, 50% as carbohydrate, and 37% as fat) for 3–6 d. They were then provided with 2 isocaloric weight-loss diets (8.3 MJ/d), which consisted of either high protein and low carbohydrate (HPMC1, containing 29% of total energy as protein, 5% as carbohydrate, and 66% as fat) or high protein and moderate carbohydrate (HPMC1, containing 28% of total energy as protein, 35% as carbohydrate, and 37% as fat). Each weight-loss diet was supplied for 21 d in a randomized crossover design. All meals were of a similar energy density of between 5.7–6.0 MJ/kg (22).

**Trial 2.** Participants (n = 14) spent 1 wk consuming the standard weight maintenance diet (MTD2; fed at 1.5 × BMR, containing 13% of total energy as protein, 52% as carbohydrate, and 35% as fat). They then received 21 d of the nonstarch polysaccharide-enriched diet (NSP) at weight maintenance energy intake, containing 13% of total energy as protein, 50% as carbohydrate, and 37% as fat. Finally, all participants spent 21 d consuming a high-protein and moderate-carbohydrate weight-loss diet (HPMC2; containing 30% of total energy as protein, 40% as carbohydrate, and 30% as fat) that contained the UK recommended minimum amount of dietary fiber. Further details of trial 2 can be found in (23) (Supplemental Tables 1, 2 and Supplemental Figure 1).

**Trial 3.** Following 1 wk of consuming the body weight maintenance diet (MTD3; fed at 1.5 × BMR, containing 15% of total energy as protein, 55% as carbohydrate, and 30% as fat), participants (n = 18) were randomized (crossover design) for consumption of 2 weight-loss diets: a normal-protein weight-loss diet (NPWL1; containing 15% of total energy as protein, 55% as carbohydrate, and 30% as fat) for 10 d or a high-protein, moderate-carbohydrate weight-loss diet (HPMC3; containing 30% of total energy as protein, 40% as carbohydrate, and 30% as fat) for 10 d. Weight-loss diets were fed at 1 × BMR as 3 meals/d and prepared on a 5-d rotation menu.

**Analysis of dietary intakes**

Using the kitchen records and participants’ food diaries, the mean nutrient intakes for each participant in all of the interventions were determined by trained staff using the WinDiets Nutritional Analysis Software Suite version 1.0 (The Robert Gordon University), a computerized version of M’Cance and Widdowson’s The Composition of Foods (24). The individual daily intakes of macronutrients (carbohydrate, fat, and protein), dietary fiber, NSP, sugar, starch, total energy, and VitC of 4 d (based on residence times of 6, 7–24, 9–30, and 12–44 h for the small intestine, proximal colon, distal colon, and the rectum, respectively (25) prior to each fecal sample collection were averaged so that they could be correlated with fecal NOC levels.

**Meat, iron from meat, and nitrate from vegetables.** The food group of red meat contained fresh and processed products of beef and pork and the white meat and fish food group contained chicken, turkey, tuna, prawns and haddock (in batter). The processed meat group contained bacon rashers, ham, and sausages. The iron content for each meat product was estimated from WinDiets tables based on M’Cance and Widdowson’s The Composition of Foods (24) (Supplemental Table 3).

The nitrate content from vegetables was estimated following the method used by Santamaria et al. (15). Items were grouped into 5 categories containing either very high nitrate at >2500 mg/kg food (lettuce, beetroot, rocket, spinach), high nitrate at 1000–2500 mg/kg food (celeriac, cabbage, leek, parsley), middle nitrate at 500–1000 mg/kg food (radicchio, turnip, coleslaw), low nitrate at 200–500 mg/kg food (broccoli, carrot, cauliflower, cucumber), or very low nitrate at <200 mg/kg food (Brussels sprouts, garlic, onion, green beans, mushrooms, peas, potatoes, tomatoes). The mean nitrate content in each category was used to calculate daily nitrate intake from vegetables (i.e., 3000 mg/kg food for very high, 1750 mg/kg food for high, 750 mg/kg food for middle, 350 mg/kg food for low, and 100 mg/kg food for very low).

**Fecal collection and analysis of fecal apparent total NOC**

Fecal samples were collected from each volunteer at the end of each dietary period. Freshly voided fecal samples were maintained at 4°C for no longer than 5 h prior to freezing at −20°C. Each sample was homogenized using a stomacher and fecal water was prepared by centrifugation of homogenized fecal samples. The concentration of NOC was

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analyzed by measuring the chemical release of NO detected on a thermal energy analyzer [for detailed description, see (26)].

Statistical methods

Dietary intakes and fecal NOC data from 3 trials (each consisting of 3 diets, with different volunteers per trial) were summarized as one value per diet per volunteer. NOC concentration data were log-transformed to stabilize variance. The effect of diet order (crossover design) of the weight-loss diets in trials 1 and 3 was investigated by fitting random effect models with volunteer as random effect and with fixed effects for period, diet, and their interaction. Because the period and period x diet interaction terms were nonsignificant, period was not considered in subsequent analyses. Although all 3 trials had an MTD and a HPMC weight-loss diet in common, the third diet differed across the trials. Because this resulted in confounding between trial and diet, trial was not included as a factor when analyzing data from the combined trials. Various residual error structures (power, autoregressive, heterogeneous, uniform) were investigated where the parameters describing these error structures were allowed to differ between trials. A comparison of deviations showed that a uniform error structure with trial-specific within- and between-volunteer random variation gave the best fit and this structure was used for all random effect models presented.

To investigate which dietary intake variables were responsible for changes in fecal NOC, log NOC was regressed on one or more dietary variables using the random error structure described above. To ensure biological plausibility, these models were constructed such that one or more dietary variables that are known to influence NOC formation were always included. These will be referred to as base variables and were selected as follows. First, meat is known to lead to NOC formation and the candidates for representing meat were the intakes of: meat, red meat, white meat, processed meat, and meat iron. Of these, red meat gave the best fit (both in simple and multiple regression models). Second, nitrate and VitC were also regarded as base variables and were always considered together, either as 2 separate terms or as a ratio. Finally, energy intake was regarded as a base variable, because changes in energy intake were part of the design of the weight-loss interventions. As an alternative to energy intake, body weight change was also considered but was found to be nonsignificant. A second alternative where dietary variables were corrected for energy density (g/MJ) was also explored, but this gave consistently worse fit in multiple regression models. Models with one or more base variables (namely red meat, nitrate and VitC, energy) and one additional variable were fitted, and goodness-of-fit was assessed by Akaike’s Information Criterion (AIC). AIC allows for the comparison of non-nested models, with a lower AIC corresponding to a better fit. Although it does not allow for formal testing of significant differences between models, as a rule of thumb, if the AIC of model k exceeds the minimum AIC of all models considered by <2 units, then model k receives substantial support; if it exceeds the minimum AIC by 2–7 units, it has considerably less support, and if it exceeds the minimum AIC by >10 units it has essentially no support (27).

Problems were encountered with multicollinearity, where inclusion of negatively correlated dietary variables resulted in unstable estimated slopes of opposite signs. Therefore, variance inflation factors (VIF) were calculated as $VIF_j = (1 - R_{j}^2)^{-1}$, where $R_j^2$ is the coefficient of determination obtained from regressing $X_j$ on the remaining $p-1$ explanatory variables. Only models with $P < 0.10$ for all slopes and $VIF_j < 5$ for all $X_j$ (28) were regarded as relevant.

All data were analyzed in GenStat 13th ed, release 13.1 (VSN International). $P < 0.05$ was regarded significant and $P < 0.10$ was regarded as a tendency for significance.

Results

The apparent total NOC concentrations in fecal waters obtained from obese men from 3 dietary intervention trials are summarized in Table 1. Fecal NOC were higher ($P < 0.030$) when participants consumed the weight-loss diets compared with the weight-maintenance diets. The variation observed in fecal NOC was considerable but comparable with the published literature (8,29).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Diet</th>
<th>NOC</th>
<th>Log NOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MTD1</td>
<td>408 ± 214</td>
<td>2.56d</td>
</tr>
<tr>
<td>1</td>
<td>HPLC1</td>
<td>1450 ± 688</td>
<td>3.09a</td>
</tr>
<tr>
<td>1</td>
<td>HPMC1</td>
<td>1745 ± 1041</td>
<td>3.15a</td>
</tr>
<tr>
<td>2</td>
<td>MTD2</td>
<td>244 ± 134</td>
<td>2.29f</td>
</tr>
<tr>
<td>2</td>
<td>NSP</td>
<td>277 ± 100</td>
<td>2.41f</td>
</tr>
<tr>
<td>2</td>
<td>HPMC2</td>
<td>866 ± 342</td>
<td>2.89b</td>
</tr>
<tr>
<td>3</td>
<td>MTD3</td>
<td>343 ± 175</td>
<td>2.48a</td>
</tr>
<tr>
<td>3</td>
<td>HPMC3</td>
<td>631 ± 293</td>
<td>2.75c</td>
</tr>
<tr>
<td>3</td>
<td>NPWL</td>
<td>454 ± 217</td>
<td>2.62d</td>
</tr>
<tr>
<td>3</td>
<td>SED</td>
<td>0.10</td>
<td></td>
</tr>
</tbody>
</table>

$P_{diet}^{2} < 0.001$

$1$ Values are mean ± SD, $n = 16$ (trial 1), 14 (trial 2), or 18 (trial 3) different participants per diet. Means without a common letter differ, $P < 0.05$ (post hoc t test). The maximum SED is presented. HPMC (1, 2, 3), high-protein, moderate-carbohydrate diet (trial 1, 2, 3); HPLC1, high-protein, low-carbohydrate-weight-loss diet; MTD (1, 2, 3), maintenance diet (trial 1, 2, 3); NOC, N-nitroso compound; NPWL, normal-protein, weight-loss diet; NSP, nonstarch polysaccharide diet; SED, standard error of difference.

$2$ Log NOC were analyzed with diet as fixed effect allowing for trial-specific variation within and between volunteers.

Regression of fecal NOC on single dietary intake variables.

To investigate which dietary components (summarized in Table 2 and Supplemental Table 4) were responsible for the observed variation in fecal NOC, log-transformed NOC concentrations were regressed on each dietary component at a time, allowing for trial-specific variation within and between volunteers. All individual dietary components were associated with fecal NOC ($P < 0.001$) (Table 3).

Dietary carbohydrate intake was separated into starch and total sugars, with the latter divided into nonmilk extrinsic sugars (NMES) and other sugars. Each of these dietary components had a negative relationship with fecal NOC ($P < 0.001$) (Table 3). Dietary fiber and NSP also had a strong negative association with the fecal NOC concentration ($P < 0.001$) (Table 3). Dietary fat and protein intakes had only weak associations with fecal NOC (indicated by high AIC) (Table 3). For the various forms of meat ingested, red meat intake had the strongest positive association with fecal NOC (Table 3). Between 36 and 66% of meat consumed in each diet was red meat. Total meat, meat iron, processed meat, and white meat intakes were also strongly correlated with fecal NOC concentrations ($P < 0.001$) (Table 3).

Nitrate intake from vegetables were positively correlated with fecal NOC ($P < 0.001$), but this was weak based on AIC. In contrast, VitC intake had a stronger but negative association with fecal NOC ($P < 0.001$). As a consequence, the NO3:VitC ratio was positively correlated with fecal NOC ($P < 0.001$) (Table 3).

Energy intake was negatively correlated with fecal NOC ($P < 0.001$), whereas body weight change as result of lower energy intake was not associated with fecal NOC ($P = 0.50$; AIC = 103.3) (results not shown).

Regression of fecal NOC on multiple dietary intake variables.

Many of the dietary components were strongly correlated. Positive correlations were observed among the various
carbohydrate intakes and among meats, whereas between these 2 food groups, negative associations were observed (Supplemental Table 5). This may result in spurious findings where a significant association between log NOC and intake variable X is actually driven by a true dietary precursor of NOC that is strongly correlated with X. To avoid this problem, multiple regression models were developed that included dietary variables known to influence endogenous NOC formation (referred to as base variables). The base variables were red meat, nitrate, and VitC. The latter 2 variables were incorporated as the ratio of NO3:VitC because it is more biologically relevant and also gave a better model fit. In addition, energy was also regarded as a base variable, because changes in energy intake were part of the design of the weight-loss diet interventions.

When all base variables were simultaneously assessed within one regression model (red meat + NO3:VitC + energy), the association with fecal NOC became much stronger with a greatly improved model fit (AIC = 26.4) (Table 4). Nonetheless, from these regression models (AIC = 19.8) (Table 4). The estimated slopes for red meat, energy, NMES, and NSP were similar across the test diets (Table 4). Although the increased NSP ratio contributed strongly, approximately one-half of the predicted difference in log NOC was attributed to reduced energy and NSP intakes (Fig. 1).

The differences between a MTD and a HPMC diet are rather complex, with a difference in total energy consumption combined with a shift in proportion of macronutrients. To separate the impact of energy intake and macronutrient composition, we first compared a MTD with a NPWL diet, where total energy intake was decreased but the macronutrient balance was similar. The decreased energy intake was the main predictor for increased NOC in feces (Table 5). The differences between a MTD and a HPMC diet are rather complex, with a difference in total energy consumption combined with a shift in proportion of macronutrients. To separate the impact of energy intake and macronutrient composition, we first compared a MTD with a NPWL diet, where total energy intake was decreased but the macronutrient balance was similar. The decreased energy intake was the main predictor for increased NOC in feces (Table 5). The differences between a MTD and a HPMC diet are rather complex, with a difference in total energy consumption combined with a shift in proportion of macronutrients. To separate the impact of energy intake and macronutrient composition, we first compared a MTD with a NPWL diet, where total energy intake was decreased but the macronutrient balance was similar. The decreased energy intake was the main predictor for increased NOC in feces (Table 5). The differences between a MTD and a HPMC diet are rather complex, with a difference in total energy consumption combined with a shift in proportion of macronutrients. To separate the impact of energy intake and macronutrient composition, we first compared a MTD with a NPWL diet, where total energy intake was decreased but the macronutrient balance was similar. The decreased energy intake was the main predictor for increased NOC in feces (Table 5).

### TABLE 2

<table>
<thead>
<tr>
<th>Trial Diet</th>
<th>Energy</th>
<th>Protein</th>
<th>Fat</th>
<th>Carbohydrate</th>
<th>Sugar</th>
<th>NMES</th>
<th>NSP</th>
<th>VitC</th>
<th>Nitrate</th>
<th>Red meat</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTD1</td>
<td>12.1±5</td>
<td>89±11</td>
<td>121±16</td>
<td>378±46</td>
<td>132±15</td>
<td>76±14</td>
<td>22±5</td>
<td>143±49</td>
<td>116±42</td>
<td>32±9</td>
</tr>
<tr>
<td>HPLC1</td>
<td>8.0±4</td>
<td>137±6</td>
<td>142±7</td>
<td>22±2</td>
<td>19±2</td>
<td>9±3</td>
<td>9±1</td>
<td>173±16</td>
<td>443±54</td>
<td>191±40</td>
</tr>
<tr>
<td>HPMC1</td>
<td>8.4±2</td>
<td>141±2</td>
<td>82±5</td>
<td>184±2</td>
<td>59±12</td>
<td>37±3</td>
<td>13±2</td>
<td>72±15</td>
<td>90±16</td>
<td>165±35</td>
</tr>
<tr>
<td>HPMC2</td>
<td>13.4±6</td>
<td>165±12</td>
<td>127±16</td>
<td>431±50</td>
<td>159±22</td>
<td>101±13</td>
<td>30±6</td>
<td>238±33</td>
<td>124±30</td>
<td>29±7</td>
</tr>
<tr>
<td>HPMC3</td>
<td>13.5±7</td>
<td>101±13</td>
<td>138±21</td>
<td>421±54</td>
<td>258±37</td>
<td>174±21</td>
<td>45±6</td>
<td>202±34</td>
<td>276±72</td>
<td>72±23</td>
</tr>
<tr>
<td>NPWL</td>
<td>8.8±1</td>
<td>153±20</td>
<td>72±9</td>
<td>219±31</td>
<td>113±15</td>
<td>90±11</td>
<td>18±2</td>
<td>145±18</td>
<td>112±20</td>
<td>158±28</td>
</tr>
</tbody>
</table>

The decreased energy intake was the main predictor for increased NOC in feces. The model predicted a difference of 0.40 log units, 81% of the observed difference (model 4, Table 4). Although the increases in red meat intake and the NO3:VitC ratio contributed strongly, approximately one-half of the predicted difference in log NOC was attributed to reduced energy and NSP intakes (Fig. 1).

The differences between a MTD and a HPMC diet are rather complex, with a difference in total energy consumption combined with a shift in proportion of macronutrients. To separate the impact of energy intake and macronutrient composition, we first compared a MTD with a NPWL diet, where total energy intake was decreased but the macronutrient balance was similar. The decreased energy intake was the main predictor for increased NOC in feces. The model predicted a difference of 0.40 log units, 81% of the observed difference (model 4, Table 4). Although the increases in red meat intake and the NO3:VitC ratio contributed strongly, approximately one-half of the predicted difference in log NOC was attributed to reduced energy and NSP intakes (Fig. 1).

### TABLE 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Slope</th>
<th>SE</th>
<th>P</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, MJ/d</td>
<td>-0.0720</td>
<td>0.0064</td>
<td>&lt;0.001</td>
<td>52.9</td>
</tr>
<tr>
<td>Carbohydrate, g/d</td>
<td>-0.0017</td>
<td>0.0001</td>
<td>&lt;0.001</td>
<td>31.2</td>
</tr>
<tr>
<td>Starch, g/d</td>
<td>-0.0028</td>
<td>0.0002</td>
<td>&lt;0.001</td>
<td>51.0</td>
</tr>
<tr>
<td>Sugars, g/d</td>
<td>-0.0032</td>
<td>0.0002</td>
<td>&lt;0.001</td>
<td>31.0</td>
</tr>
<tr>
<td>NMES, g/d</td>
<td>-0.0048</td>
<td>0.0004</td>
<td>&lt;0.001</td>
<td>47.1</td>
</tr>
<tr>
<td>Other sugars, g/d</td>
<td>-0.0048</td>
<td>0.0005</td>
<td>&lt;0.001</td>
<td>62.8</td>
</tr>
<tr>
<td>Dietary fiber, g/d</td>
<td>-0.0194</td>
<td>0.0018</td>
<td>&lt;0.001</td>
<td>56.1</td>
</tr>
<tr>
<td>NSP, g/d</td>
<td>-0.0216</td>
<td>0.0019</td>
<td>&lt;0.001</td>
<td>55.3</td>
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<tr>
<td>Protein, g/d</td>
<td>0.0041</td>
<td>0.0006</td>
<td>&lt;0.001</td>
<td>88.4</td>
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<tr>
<td>Fat, g/d</td>
<td>-0.0041</td>
<td>0.0006</td>
<td>&lt;0.001</td>
<td>87.9</td>
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<tr>
<td>Meat, g/d</td>
<td>0.0015</td>
<td>0.0001</td>
<td>&lt;0.001</td>
<td>57.2</td>
</tr>
<tr>
<td>Red meat, g/d</td>
<td>0.0028</td>
<td>0.0002</td>
<td>&lt;0.001</td>
<td>52.4</td>
</tr>
<tr>
<td>White meat, g/d</td>
<td>0.0018</td>
<td>0.0002</td>
<td>&lt;0.001</td>
<td>76.3</td>
</tr>
<tr>
<td>Processed meat, g/d</td>
<td>0.0027</td>
<td>0.0003</td>
<td>&lt;0.001</td>
<td>68.3</td>
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<tr>
<td>Meat iron, mg/d</td>
<td>0.1910</td>
<td>0.0198</td>
<td>&lt;0.001</td>
<td>83.6</td>
</tr>
<tr>
<td>VitC, mg/d</td>
<td>-0.0032</td>
<td>0.0003</td>
<td>&lt;0.001</td>
<td>66.1</td>
</tr>
<tr>
<td>NO3, mg/d</td>
<td>0.0010</td>
<td>0.0002</td>
<td>&lt;0.001</td>
<td>92.2</td>
</tr>
<tr>
<td>NO3:VitC ratio</td>
<td>0.2961</td>
<td>0.0273</td>
<td>&lt;0.001</td>
<td>63.3</td>
</tr>
<tr>
<td>Red meat:NSP ratio</td>
<td>0.0353</td>
<td>0.0027</td>
<td>&lt;0.001</td>
<td>38.4</td>
</tr>
</tbody>
</table>

1 Random effects linear regression of log NOC (µg/L) on one dietary variable at a time, allowing for trial-specific random variation between and within volunteers; 48 obese volunteers, 3 diets/volunteer. AIC, Akaike’s Information Criterion; NMES, nonmilk extrinsic sugar; NOC, N-nitroso compound; NSP, nonstarch polysaccharide diet; VitC, vitamin C.
Our results indicate that nondigestible but fermentable carbohydrates such as NSP have a potential to protect against increased NOC formation when consuming high-meat diets (maximum 216 g/d). Reports in the literature suggest that the incorporation of fiber into high-red meat diets might alter NOC formation. For example, a study using 420 g/d red meat found a significant reduction in fecal NOC when supplementing with an additional 17 g/d of NSP (20). In contrast, other studies using 600 g/d red meat diets found that increasing resistant starch (hence increasing protein and red meat intake at a similar energy intake), energy became the least important contributor (Fig. 1). Increased red meat intake became the most important predictor for increased fecal NOC, but an increased NO3:VitC ratio and a decreased NSP intake also contributed substantially.

Discussion

In our experiments, obese men consumed different weight-loss diets with varying protein and meat contents and their stool samples analyzed for NOC. The detailed dietary intake data allowed us to assess the contribution of different dietary components to endogenous formation of NOC in the human gut. It also allowed us to study the individual contributions and combined impact of various dietary components on endogenous NOC formation.

Dietary intakes of nitrate and nitrite have long been suggested to contribute to endogenous NOC formation in the human gut. Shifting from a low-nitrate diet to a high-nitrate diet significantly increased fecal and urinary NOC in humans (16,30). Our study diets ranged from moderate (80 mg/d) to high nitrate (443 mg/d) intakes. Comparing the HPMC diets of trials 1 and 2, we found that a lower nitrate intake coincided with higher fecal NOC in individuals in trial 1 compared with trial 2. This may be due to the fact that intakes of potentially protective dietary components were also lower in diet HPMC1 (e.g., 2.5-fold lower VitC). VitC has been found to counteract the oxidation of dietary nitrate to nitrite performed by oral bacteria. Nitrite acts as a precursor for NOC formation in the stomach (16,17,31,32). Our data confirm a positive association of endogenous NOC formation with dietary nitrate and a negative association with VitC. When we simultaneously examined both nitrate and VitC intakes (either as separate variables or as the NO3:VitC ratio), this association became stronger, confirming their interlinked role in endogenous NOC formation. NOC formed in the stomach are thought to be nitrosamines from direct nitrosation of dietary secondary amines and nitroso-thiols, which can act as precursors and NO donors in the lower gut (19), hence explaining a relationship between stomach conditions and fecal NOC concentrations.

The association between high red meat intake and increased risk of colorectal cancer is well established (2). One proposed mechanism by which red meat might increase cancer risk involves the potential to trigger endogenous formation of carcinogenic NOC in the human gut (8,12,29). Our results confirm a strong association between red meat intake and fecal NOC, although this does not prove that red meat intake is a trigger of endogenous NOC formation. Our study focused on obese men consuming either MTD or high-protein, weight-loss diets. High-protein diets, which are often high in meat, are a well-established way to control body weight (22). Simultaneous restriction in carbohydrate intake further aids weight loss and causes marked shifts in gut bacterial populations and bacterial fermentation products (26). We show that these meat-based, high-protein weight-loss diets significantly increase fecal concentrations of NOC and that energy intake was negatively correlated with fecal NOC. However, body weight change was not associated with fecal NOC, probably because weight loss was relatively minor and variable between participants (mean weight loss, 160 ± 56 g/d). Endogenous NOC formation was further enhanced by decreased intake of NMES and NSP, which had a significant effect even after the known dietary contributors (red meat, nitrate, VitC) and energy intake were taken into account. This suggests that NMES have an additional, energy-independent effect on endogenous NOC formation, but where and how this protection occurs in the upper intestinal tract requires further investigation.

Our results indicate that nondigestible but fermentable carbohydrates such as NSP have a potential to protect against increased NOC formation when consuming high-meat diets (maximum 216 g/d). Reports in the literature suggest that the incorporation of fiber into high-red meat diets might alter NOC formation. For example, a study using 420 g/d red meat found a significant reduction in fecal NOC when supplementing with an additional 17 g/d of NSP (20). In contrast, other studies using 600 g/d red meat diets found that increasing resistant starch
from 14 to 51 g/d (10) or increasing NSP from 30 to 45 g/d did not significantly alter fecal NOC. One explanation for these apparent contradictory observations may be that any protective effect of fermentable carbohydrates is masked when a large excess amount of red meat is consumed. Interestingly, in the present study, an increase in dietary NSP from 30 to 45 g/d did not significantly reduce fecal NOC, because it co-occurred with an increase in red meat intake from 29 to 75 g/d. Therefore, not only the absolute amounts of red meat and fiber intake but also their ratio may influence endogenous NOC formation. In the current study, we were unable to assess the possible effect of a red meat:NSP ratio, because this was strongly correlated with the NO3/VitC ratio. Future human studies will need to include diet designs tailored to study the red meat:fiber ratio and the potential protective effect of fermentable carbohydrate against meat-induced endogenous NOC formation.

Evidence from rodent studies suggests a beneficial effect of fermentable carbohydrate to ameliorate the harmful effects of high-protein feeding on colonic tissues. DNA damage and promutagenic DNA-adducts in colonicocytes have been shown to be higher in high-protein and high-red meat feeding (33–35), and these effects were attenuated by the addition of resistant starch. In a human study, whereas meat consumption was higher in colorectal cancer patients than in controls, the DNA adducts in colonic tissue were not associated with meat intake but were inversely associated with NSP intake (36). Even though NOC concentrations were not determined in these studies, they provide a possible of the DNA adducts that are specific to DNA-methylating agents such as NOC. The ultimate link between diet, endogenously formed NOC, and causation and prevention of DNA damage in animals and humans is still missing.

The published literature has focused on red meat and meat iron as the major factors driving endogenous formation of NOC (8,12–14). A prediction model for endogenous formation of NOC has been suggested based solely on the estimation of an individual's meat iron intake (11). Our data clearly demonstrate, however, that besides red meat, other dietary component intakes, including that of NO3, VitC, and NSP or NMES as well as energy, play important additional roles in influencing the endogenous formation of NOC in the human gut. Indeed, using the published prediction model (11), based on meat iron intake alone, we were able to predict only 55% of the observed change in fecal NOC concentrations when comparing a MTD to a high-protein, weight-loss diet. In contrast, our current proposed model predicted 81% of the observed change, illustrating the importance of including alterations in VitC, nitrate, energy, and NSP intakes. The heme-alone model has been used to predict endogenous NOC formation in a large-scale European study, but no relationship between endogenous NOC and cancer at any site was found in a cohort of >23,000 people (37). Based on our current findings, we think that future studies would benefit from more detailed dietary assessment and more complex prediction models for endogenous NOC formation. The current data are limited to obese men consuming weight maintenance or weight-loss diets and our results need to be confirmed in wider populations. If an increase in potentially harmful food components such as red meat or nitrate co-occurs with a decrease in possible protective food items, including VitC and fiber, their effects on the endogenous formation of NOC could be accelerated. Predicting the relative contributions of several dietary components to endogenous NOC formation is therefore of critical importance to better describe the complex relationship among diet, endogenous formation of NOC, and the possible impact on human health.

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Literature Cited


