Red & cured meat and colon cancer: Should we become vegetarians, or can we make meat safer?

Denis Corpet
Pierre F., Santarelli RL, Bastide NM, Guéraud F, Vendeuvre JL, Taché S, Naud N, Attaix, D.
ToxAlim - INRA - Institut National Recherche Agronomique
ENVT - Ecole Nationale Vétérinaire Toulouse - France

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  DGER-ENVT (Natl. Vet. School, France).
Is this true?
Continuous Update Project  
WCRCF & AICR - May 2011

- The WCRF-AICR Continuous Update Project Panel agreed that the recent evidence was consistent with the conclusion of the Second Expert Report (2007):
- Red meat and processed meat are convincing causes of colorectal cancer.
- Recommendations for meat eaters are:
  - to limit fresh red meat intake to less than 500 g/week
  - to avoid processed meat (0 g per week).
Meat intake is not the only lifestyle factor that modulates colorectal cancer.

Summary estimates of Relative Risk (RR) from cohort studies meta-analysis (WCRF 2007)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Evidence strength</th>
<th>RR</th>
<th>Signif.</th>
<th>Percent change</th>
<th>per</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal Fatness</td>
<td>++</td>
<td>1.30</td>
<td>*</td>
<td>+ 30%</td>
<td>0.1 W-to-H</td>
</tr>
<tr>
<td>Red meat</td>
<td>++</td>
<td>1.29</td>
<td>*</td>
<td>+ 29%</td>
<td>100 g/d</td>
</tr>
<tr>
<td>Garlic</td>
<td>+</td>
<td>0.73</td>
<td>*</td>
<td>- 27%</td>
<td>high vs. low</td>
</tr>
<tr>
<td>Alcohol</td>
<td>++</td>
<td>1.27</td>
<td>*</td>
<td>+ 27%</td>
<td>30 g/d</td>
</tr>
<tr>
<td>Smoking</td>
<td>++</td>
<td>1.25</td>
<td>*</td>
<td>+ 25%</td>
<td>ever vs. never</td>
</tr>
<tr>
<td>Processed meat</td>
<td>++</td>
<td>1.21</td>
<td>*</td>
<td>+ 21%</td>
<td>50 g/d</td>
</tr>
<tr>
<td>Body fatness</td>
<td>++</td>
<td>1.15</td>
<td>*</td>
<td>+ 15%</td>
<td>5 kg/m²</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>+</td>
<td>0.90</td>
<td>*</td>
<td>- 10%</td>
<td>10 g/d</td>
</tr>
<tr>
<td>Adult attained height</td>
<td>++</td>
<td>1.09</td>
<td>*</td>
<td>+ 9%</td>
<td>5 cm</td>
</tr>
<tr>
<td>Milk</td>
<td>+</td>
<td>0.94</td>
<td>NS</td>
<td>- 6%</td>
<td>serving/d</td>
</tr>
<tr>
<td>Calcium</td>
<td>+</td>
<td>0.95</td>
<td>MS</td>
<td>- 5%</td>
<td>500 mg/d</td>
</tr>
</tbody>
</table>

Evidence strength:
++ Convincing
+ Probable

Five Hypotheses on Meat & Colorectal Cancer

- **H1**: Red meat contains myoglobin with **heme iron**

- **H2**: Cured meat contains nitrate & nitrite => N-nitrosated compounds (NOC)

- **H3**: Cooking at a high temperature => **Heterocyclic Aromatic Amines** and Polycyclic Aromatic Hydrocarbons

- **H4**: Fat => excess calories & bile acids

- **H5**: Meat may lack protecting agents (e.g., calcium, phytochemicals)
Method: Rats given a colon carcinogen

DMH, AOM, PhIP, MNU, MNNG...

No spontaneous colon cancer in rats but easy to induce ACF, MDF & cancers

Tumor development & pathology are similar in rats and in humans

Rat model with precancer lesions

ACF, Aberrant Crypt Foci
Methylene Blue staining x40, 15d after carcinogen initiation
Correlation with cancer, not 100%
(Bird, Cancer Let. 1987)
Ki-ras mutation

MDF, Mucin Depleted Foci
HIDAB staining x40, high iron diamine Alcian blue
100d after carcinogen initiation
MDF-cancer correlation >> ACF
(Caderni, Cancer Res. 2002)
Apc mutation (Femia, 2007)
Rat Model → First evidence of red meat & heme promotion of colon carcinogenesis:

In a calcium-depleted diet, Beef meat & Black pudding (blood sausage) promote Mucin Depleted Foci (& ACF)
More heme = more MDF

Beef meat & Black pudding raise fecal & urinary markers of fat peroxidation:
TBARs, DHN-MA, cytotoxicity
More heme = more peroxides

Pierre et al., J.Nutr. 2004

Dietary Calcium normalizes beef meat effects

⇒ Calcium fully suppressed beef-induced promotion

Fat peroxides: MDA genotoxic & cytotoxic

⇒ Calcium fully suppressed beef-induced lipoperoxidation

Pierre et al., British Journal of Nutrition (2008)
Published mechanistic studies suggest that heme-induced fat peroxides promote colon cancer by selection of \emph{apc} mutated cells in the colonic mucosa.

In all our previous rats studies, carcinogenesis promotion by meat or heme was associated with fat peroxidations biomarkers:
- Fecal water TBARs
- Fecal water cytotoxicity
- Urinary DHN-MA (metabolite of 4-hydroxynonenal)

\cite{Pierre et al., 2003, 2004, 2006, 2007; Santarelli et al., 2008}

The agents that decrease the level of these biomarkers also suppress carcinogenesis in rats.

**How can haem & lipoperoxides promote cancer?**

We guess it is by selection of cancer cells.

Similar to selection of drug resistant bacteria by antibiotics.

1. 
   A bunch of bacteria, including a resistant variety...

2. 
   ... get bathed in antibiotics. Most of the normal bacteria die.

3. 
   The resistant bacteria multiply and become more common.

4. 
   Eventually, the entire infection evolves into a resistant strain.
**Freeze-dried oxidized cooked ham**
given to initiated rats promotes precancer MDF and
increases markers of lipoperoxidation & cytotoxicity

![Graphs showing MDF and ACF/10 levels in control vs. ham groups.](image)

**Pierre et al., Nutr. & Cancer, 2010**

D.E. Corpet – iCoMST - Ghent - 2011

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**Design of a short-term study in rats**
given 16 models of cured meat

- Anaerobic packaging & Five day air exposure
- Added Nitrite
- Zero ppm
- N 150 ppm
- Cooked Raw
- 70°C 50°C

Short-term study of 2x2x2x2 factors
= 16 models of cured meat.
End-points: Early fecal and urinary biomarkers used as screening tools

**Principal component analysis**
of biomarker data,
to choose 4 cured meat models
→ 100 d carcinogenesis study

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![Diagram showing principal component analysis](image)
Dark Cooked Nitrited Oxidized = DCNO
Dark Raw Zero-Nitrite Oxidized = DCZO
Dark Raw Zero-Nitrite Anaerobic = DRZA
Dark Cooked Nitrited Anaerobic = DCNA

Given for 100 days to carcinogen-initiated rats

CON DRZA DCZO DCNA DCNO

Effect of a cured meat diet on MDF and NOC formation in the colon of rats 106 days after carcinogen injection (values are means ± SD, n = 10. * significantly different from control P < 0.05).

But if No Oxygen or No Nitrite
DCNA & DCZO → No promotion

Fecal N-nitrosated compounds clearly associated with promotion

DCNO model cured meat (Dark, Cooked, Nitrite-treated and Oxidized high-heme meat) promotes colon carcinogenesis and increases fecal end products of lipid peroxidation in rats (Santarelli et al., Cancer Prevention Research, July 2010).
Unpublished Prevention Study in Rats

Aim: to prevent the promoting and pro-oxidant effects of cured meat with a heme iron binding-additive or an antioxidant-additive.

Calcium carbonate (150µmol/g) or α-tocopherol (0.05%) added to the model cured meat diet DCNO, and given for 100 days to rats pretreated with a carcinogen. Colons were scored for preneoplastic MDF.
Results 1: Prevention of Cured Meat MDF Promotion in Rats

In rats, cured meat DCNO increased the number of MDF/colon after feeding for 100d (p=0.01)

Calcium and α-tocopherol fully normalized the number of MDF/colon (p=0.01)

Full Data not shown before publication

Results 2: Prevention of Cured Meat-Induced Fecal Peroxidation in Rats

In rats, cured meat increased the fecal TBARs (fat peroxides)

Calcium, but not tocopherol, reduced fecal TBARs & cytotoxicity, and urinary DHN-MA, in cured meat-fed rats (only TBARs data are shown)

Nitroso-compounds (NOC) assays are ongoing

Full Data not shown before publication
Unpublished Prevention Study in Volunteers

- In a cross-over trial, 17 healthy volunteers were given the model cured meat (DCNO = 160g/d “ham” for 4 days), as such or supplemented with calcium carbonate (1g/d) or with α-tocopherol (80mg/d).

Stool samples assayed for TBARs and NOC (ongoing)

<table>
<thead>
<tr>
<th>Inclusion</th>
<th>Run-in 7 d</th>
<th>First period 4 d</th>
<th>Wash Out 3 d</th>
<th>Second period 4 d</th>
<th>Wash Out 2 d</th>
<th>Third period 4 d</th>
</tr>
</thead>
</table>

First human data fit rats data

TBARs increased in stools of 17 volunteers given cured meat compared with meat-free period (Wilcoxon P<0.05).

Calcium and α-tocopherol supplementation fully normalized fecal TBARS in volunteers given cured meat (P<0.05).

Fecal nitroso-compounds (NOC, ATNC) assays are ongoing

Effect of cured meat diets on fecal biomarkers (TBARs) in stools of volunteers after 4 days on experimental diets (values are means ± SEM, n = 17)

* Significantly different from meat free period, P < 0.05
° Significantly different from DCNO period, P < 0.05

Full Data not shown before publication
**Conclusion of the unpublished study**

- Promotion of colon carcinogenesis in rats by cured meat (DCNO) was suppressed by dietary calcium and by α-tocopherol. Calcium normalized associated fecal and urinary biomarkers in rats (TBARs, cytotoxicity, DHN-MA).
- Dietary calcium and α-tocopherol normalized fecal TBARs in human volunteers given cured meat.
- Many people are reluctant to change their diet: the advice “avoid processed meat” is badly adhered to. The addition of specific agents to cured meat may provide a new way to prevent colorectal cancer… and to move towards a fair society?

**Life Expectancy at Birth, and Disability-Free Life Expectancy (DFLE)**


Slide from M. Marmot, 2010
Health inequalities

- The poor live shorter lives in good health than the rich. Colorectal cancer is a part of the inequality burden. Red meat & cured meat are a part of the colorectal cancer burden.
- Less educated people do not follow advices about smoking, exercise, sensible drinking and healthy eating.
- But are they really free to do so? (Marmot, 2010)
- Our responsibility is to make a safer meat:

  More ethical, an easier, to change the food than to change the consumer
Go vegan … or make your meat safer!

Potential paths for a safer cured meat:
- Change diet (e.g., eat more calcium)
- Change process (e.g., without O₂ or NO₃)
- Use new additives (e.g., tocopherol)

Sorry, but no recipe is available yet!
Is this true?