Fermented Meats and Implications in Human Health: The Microbiome Connection

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FHCRC

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Overview

- Population-level health trends associated with meat consumption
- What is fermented meat?
- How does the microbiome play a role in meat fermentation?
- Ingestion of fermented meat and microbial mechanisms related to human health trends
  - Microbial sources of precursors of Nitrite ($\text{NO}_2$), Nitric Oxide (NO), and N-nitroso compounds (NOC)
Meat consumption increased

US consumption > EU

Red meat > poultry and fish

Processed Meat Consumption and Cancer

• **WCRF/AICR 2007**

Reviewed 16 cohort studies and 71 case-control studies

Positive “moderate” association between the consumption of red and processed meats and colorectal cancer (RR<2)

Probable association between fermented fish and nasopharyngeal cancers

• **Recommendation:**
  Reduce intake of red meat to 500g/week and do not consume processed meats

• **Methodological problems:** needed to quantify processed meats more accurately
# Processed meat-related Compounds Associated with Colorectal Cancer

## Prospective study

**NIH-AARP Diet and Health Study, n=500,000**

- Food Frequency Questionnaire, Risk Factor Questionnaire, updated databases for dietary nitrate + nitrite consumption + charred meats

## Heterocyclic amines

- Precursors for N-nitroso compound (NOC)

## Nitrate

- \( P_{\text{trend}} = 0.001 \)

## Nitrite

- \( P_{\text{trend}} = 0.055 \)

## Iron

- Dietary intake \( P_{\text{trend}} < 0.001 \)
- Heme Iron \( P_{\text{trend}} = 0.022 \)

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**Cross et al., 2010, Cancer Res, 70(6)**

### HR* (95% CI)

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<th>Total Iron mg/day</th>
<th>Q1 (10.8)</th>
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<td>Q2 (14.8)</td>
<td>646</td>
<td>0.91 (0.81-1.02)</td>
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<tr>
<td>Q3 (21.5)</td>
<td>539</td>
<td>0.88 (0.78-0.99)</td>
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<tr>
<td>Q4 (30.6)</td>
<td>496</td>
<td>0.81 (0.72-0.91)</td>
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<td>Q5 (36.1)</td>
<td>460</td>
<td>0.75 (0.66-0.86)</td>
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<th>Dietary Iron mg/1000 kcal</th>
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<td>Q1 (5.9)</td>
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<td>Q2 (7.2)</td>
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<td>Q3 (8.2)</td>
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<td>Q4 (9.3)</td>
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<td>Q2 (100.9)</td>
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<td>Q4 (212.6)</td>
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<td>Q5 (335.8)</td>
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<th>Nitrate ug/1000 kcal</th>
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<td>Q2 (65.3)</td>
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<td>Q3 (109.6)</td>
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<td>Q4 (169.2)</td>
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<td>Q5 (289.2)</td>
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<th>Nitrite ug/1000 kcal</th>
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<td>Q2 (33.7)</td>
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<td>Q3 (59.7)</td>
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<td>Q4 (99.9)</td>
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<td>Q5 (194.1)</td>
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What is Fermented Meat?

- Falls in the category of Processed Meat

- Processed meat is preserved by smoking curing, salting, or adding chemicals (Nitrite, Nitrate)

- Cured Meats
  - Entire pieces of raw or cooked muscle meat
  - Nitrate/nitrite as dry salt or salt solution
  - Ripening process with “drying”
  - Prosciutto

- Raw-fermented Sausages
  - Uncooked coarse mixtures of lean meats and fatty tissue in casings
  - Salt
  - Nitrate/nitrite
  - Ripening process with “drying”
  - Anaerobic fermentation: with or without “starter” culture
  - Salami
Why Ferment Meat?

• Fermented anaerobic microbial metabolism by bacteria or yeast

• Boosts acidity/reduces pathogens

• Counteract rancidity of fats

• Develop flavor and taste due to organic acids

• Texture improvement of ripened products through proteolysis and aa fermentation

• With or without salts, nitrate, nitrite, or smoking
Fermentation with Nitrite

- **Quality**
  Pink coloring -> Nitric oxide (NO) + myoglobin -> NO-myoglobin

  Flavor -> controls lipid peroxidation and fermentation end-products

- **Microbiological safety**
  Antibacterial benefits -> change in pH, high salt, and iron content

  May be species specific:
  - Gram negative enterics (*E. coli/Salmonella*)
  - *C. botulinum* affects prevents cell division in vegetative cells
  - *L. monocytogenes* in combination with prebiotic (lactate acetate)

- **Health concerns:**
  N0₂ to NO and combines with secondary and tertiary amines to form N-nitroso compounds (NOC) -> genotoxic

  Sindelar, JJ and Milkowski, AL, 2013, Nitric Oxide, 26:259
Types of Fermentation

Alcoholic \((Saccharomyces)\)
- Glucose: \(C_6H_{12}O_6 \rightarrow 2 C_2H_5OH + 2 CO_2\)

Acetic acid \((Acetobacter)\)
- Ethanol: \(C_2H_5OH + O_2 \rightarrow CH_3COOH + H_2O\)

Lactic Acid \((Leuconostoc, Lactobacillus, Streptococcus)\)
- Glucose: \(C_6H_{12}O_6 \rightarrow 2 CH_3CHOHCOOH\)
- Lactose: \(C_{12}H_{22}O_{11} + H_2O \rightarrow 4 CH_3CHOHCOOH\)

Heterolactic acid
- Glucose: \(C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2\)

Alkaline \((Bacillus)\)
- Proteins $\rightarrow$ amino acids + NH$_3$
Nitrogen metabolism

**Bacterial**

**Anaerobic**
- Dissimilatory Nitrate/Nitrite Reduction-
  \[ \text{NO}_3^- \rightarrow \text{NO}_2^- \] (Nitrate reductase)
  \[ \text{NO}_2^- \rightarrow \text{NO} \] (Nitrite reductase)

**Ammonification**
- \[ \text{NO}_2^- \rightarrow \text{NH}_3^- \rightarrow \text{NH}_4^+ \] assimilation

**Eukaryotic**

**Aerobic**
- Arginine +O\(_2\) -> NO (Nitric Oxide Synthase)

**Abiotic**
- \[ \text{NO}_2^- \rightarrow \text{NO} \] disproportionation pH<5
Microbial Mechanisms Associated with Dietary Intake of Fermented Meat

Fermented meat:
Bacterial fermentation/Disproportionation

Mouth:
Nitrate Reduction

Stomach:
Disproportionation and N-nitroso formation

Gut:
Nitric oxide and N-nitroso compound formation
Protein degradation
Lipoperoxidation/Hemin
Distribution of Bacterial Families during Ripening/Drying of Sausage

Time zero - reflects meat microbiome and most diversity

Staphylococcus, Lactobacillus, Bifidobacter, Brevibacter

Shifts towards Lactobacillus with time

Physico-chemical changes: water potential is reduced, pH is reduced, NO can be formed

Microbial Mechanisms Associated with Dietary Intake of Fermented Meat

Fermented meat: Bacterial fermentation/Disproportionation

Mouth: Nitrate Reduction

Stomach: Disproportionation and NOC formation

Gut: NO and NOC Formation
      Protein degradation
      Lipoperoxidation/Hemin
Entero-salivary NO$_3$-NO$_2$ pathway

Anti-bacterial mouth wash reduced health effects of NO$_3$ (increased BP) (Petersen et al., 2009; Free Radic Biol Med.; 46:1068)

Ingested dietary NO$_3$

Enters blood stream in the small intestine

Combines with endogenous NO$_3$

Up to 25% actively taken up by salivary gland
Nitrate Reducing Bacteria in the Oral Cavity a source of NOC Precursors

Nitric oxide synthase independent/O₂ independent pathway (Microbial)

Oral bacteria→NO₃→NO₂

Tongue microbiome from High/Low NO₂ producers

High producers more diverse

Functional genes enriched for nitrate reduction

(Hyde, E. et al.; 2014;3:e88645)
Fermented meat: 
Bacterial fermentation/Disproportionation

Mouth:
Nitrate Reduction

Stomach:
Disproportionation and NOC formation

Gut:
NO and NOC Formation
Protein degradation
Lipoperoxidation/Hemin
Nitrosation in the Stomach
Disproportionation and NOC formation

\[ \text{pH}<5 \ \text{NO}_2 \text{ disproportionates to Nitric oxide (NO)} \]

\[ \downarrow \]

Dietary proteins are decomposed to amines and amides

\[ \downarrow \]

Form N-nitroso compounds

\[ \downarrow \]

N-nitroso compounds enter the gut and hydroxylated by CYP450

\[ \downarrow \]

Alkylating agents that form DNA adducts that cause mismatch and miscoding

Amine/Amide + NO

N-nitroso compounds

\[ \text{H}_2\text{N}\text{C} \text{N}\text{H} - \text{R} + \text{O} = \text{N} \text{O}^{-} \rightarrow \text{H}_2\text{N}\text{C} \text{N}\text{H} - \text{R} + \text{O} = \text{N} \text{O}^{-} \rightarrow \text{N}-\text{nitrosoo compounds} \]
Microbial Mechanisms Associated with Dietary Intake of Fermented Meat

Fermented meat:
- Bacterial fermentation/Disproportionation

Mouth:
- Nitrate Reduction

Stomach:
- Disproportionation and NOC formation

Gut:
- NO and NOC Formation
- Protein degradation
- Lipoperoxidation/Hemin
Microbial Nitrosation in the Gut

30% of NO₃ intake makes it to the gut (Bartholemew B et al.; 1985; Good Toxicol;22:789)

Protein digestion -> amines and amides

Apparent total N-nitroso cmpds (ATNC) increased in CV vs GF mice fed NO₃ in drinking water

(Nassey et al., 1988, Ed. Chem. Toxic 26:595)

Nitrate reductase activity increases in feces along GI tract of CV rats

(Mallet et al., 1983, AEM,45:116)

N-nitroso compound producers
Gamma Proteobacteria
Peptococcus

Proline

\[ \text{Proline} + \text{nitrate} \rightarrow \text{N-nitrosoproline} \]

Intestinal bacteria

\[ \text{N-nitrosoproline} \]
We Are What We Eat: Mammals and Their Gut Microbes Cluster by Diet

Ley, RE et al., Science, 2008, 320:1647
Gut Microbiome Affects Exposure: N-nitroso Compounds

17 obese men, randomized cross-over design, 4 wks

- **HPMC**: high-protein, medium carbohydrate
- **HPLC**: high-protein, low carbohydrate

- > N-nitroso compounds

Russell et al., *Am J Clin Nutr* 2011;93:1062

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<th>M</th>
<th>HPMC</th>
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<td>Protein, %</td>
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<td>28</td>
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<tr>
<td>Fat, %</td>
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<td>Carb, %</td>
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<td>NSP, g</td>
<td>22</td>
<td>9</td>
<td>13</td>
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a,b,c significantly different p<0.001
Gut Microbiome Affects Exposure: N-nitroso Compounds

- Shift in gut microbiome and metabolites with diet
- Diet composition matters

**Maintenance diet**: High-protein/Low carbohydrate (Axis 2 (18%))

**Axis 1 (29%)**
- High-protein
- Moderate carbohydrate

HPLC diet:
- >N-nitroso compounds
- < Butyrate
- < *Roseburia*/*Eubacterium rectale*

Russell et al., *Am J Clin Nutr* 2011;93:1062
Heme Iron, Lipoperoxidation, and the Microbiome

- Heme-induced lipoperoxidation forms Malondialdehyde (MDA) -> DNA adducts genotoxic

- Rats given Hemoglobin or Hemin

- Antibiotics reduced lipoperoxidation

- Change in the microbiome?
  + Catalase; -H₂O₂; lipoperoxidation - Bifidobacteria, Lactobacillus, and Streptococcus
  Zhang et al 2013, World. J. Microbio. 29:12
  - Catalase; +H₂O₂; lipoperoxidation + Enterobacteriaceae
SUMMARY

Microbial metabolism in human systems may account for some underlying variation associated with epidemiologic trends associated with fermented meat consumption.

Although fermented meats contain compounds of nutritional benefit (iron, protein, zinc, B_{12}), consumption of large amounts may shift the gut microbiome towards metabolism that produces metabolites that affect host health.

Integration of biomarkers of meat intake, gene expression in response to fermented meat intake, individual variation in genetic disposition, and variation in the fermented meat metabolism by the gut microbiome should be considered.

An integrated approach that incorporates, both human cell line and gut microbiome in vitro studies, in silico studies, animal studies, and human dietary interventions are important.
QUESTIONS ?
Exposures and Cellular Processes Linked to Cancer

Involvement of Microbial Processes

Exposures and **microbial processes**

- DNA repair
- Carcinogen metabolism
- Hormonal regulation
- Inflammation
- Immune function
- Proliferation
- Apoptosis
- Differentiation

Adapted from WCRF/AICR 2007 Expert Report
Processed Meat Consumption and Cancer

Since 2007: supportive of trend, although ~17% RR is low;  

+ Association

CRC

20 prospective studies (Highest vs Lowest SRR=1.16)  

Colorectal adenomas

5 prospective studies (Highest vs Lowest SRR= 1.17)  

21 prospective studies (Highest vs lowest SRR= 1.18)  
(Chan et al., PLoS One 2011;6:e20456)

Null association

Liver, pancreas, breast, lung, glioma, and non-Hodgkin lymphoma