Fermented Meats and Implications in Human Health: The Microbiome Connection

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FHCRC

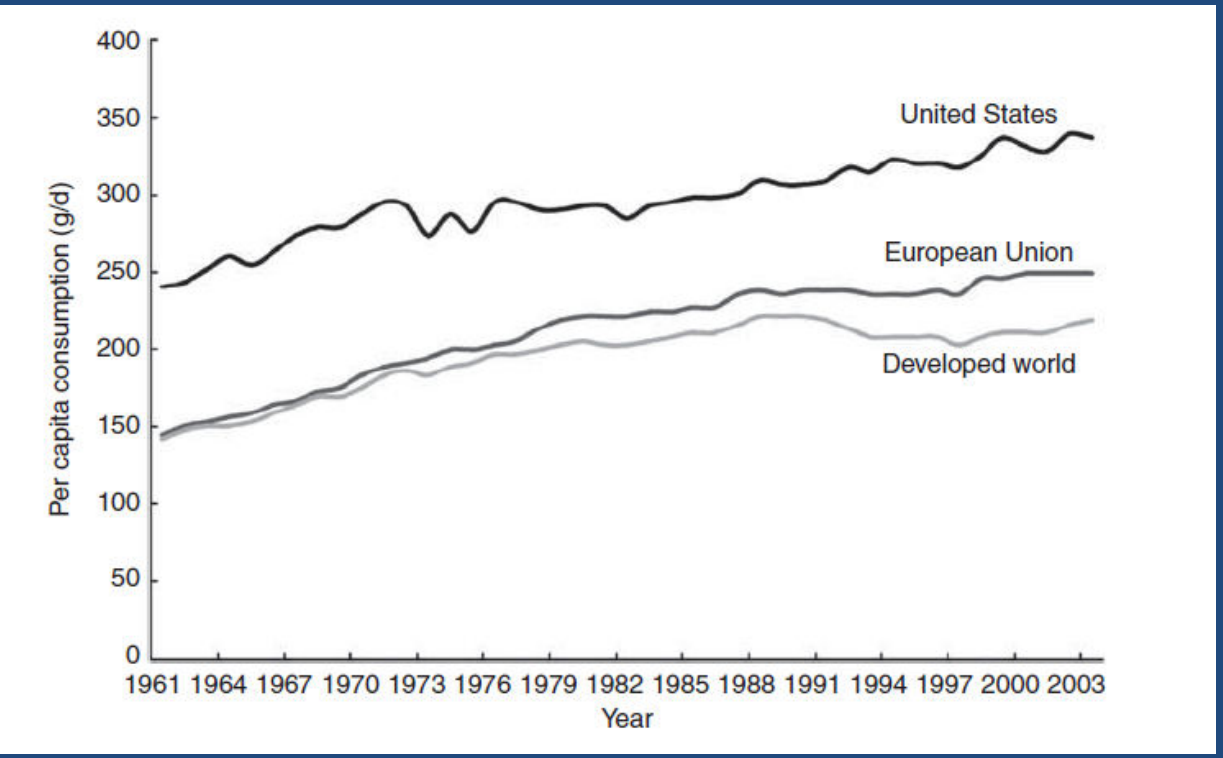
Food Technology Conference, 2015



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 (NO_2), Nitric Oxide (NO), and N-nitroso compounds (NOC)

Trends in Meat Consumption



Meat consumption increased

US consumption > EU

Red meat > poultry and fish

Daniel, CR et al.; 2011,Pub. Health Nut. ;14(4):575.

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

		HR* (95% CI)
Total Iron mg/day		
Q1 (10.8)	646	1.0
Q2 (14.8)	578	0.91 (0.81–1.02)
Q3 (21.5)	539	0.88 (0.78–0.99)
Q4 (30.6)	496	0.81 (0.72–0.91)
Q5 (36.1)	460	0.75 (0.66–0.86)
P_{trend}		<0.001
Dietary Iron mg/1000 kcal		
Q1 (5.9)	677	1.0
Q2 (7.2)	537	0.82 (0.73–0.92)
Q3 (8.2)	518	0.80 (0.70–0.90)
Q4 (9.3)	509	0.79 (0.69–0.90)
Q5 (11.4)	478	0.75 (0.65–0.87)
		<0.001
Heme Iron ug/1000 kcal		
Q1 (48.1)	468	1.0
Q2 (100.9)	508	1.00 (0.88–1.13)
Q3 (150.3)	538	1.02 (0.90–1.16)
Q4 (212.6)	577	1.07 (0.94–1.21)
Q5 (335.8)	628	1.13 (0.99–1.29)
P_{trend}		0.022
Nitrate ug/1000 kcal		
Q1 (23.9)	451	1.0
Q2 (65.3)	470	0.96 (0.85–1.10)
Q3 (109.6)	530	1.04 (0.91–1.18)
Q4 (169.2)	609	1.13 (1.00–1.29)
Q5 (289.2)	659	1.16 (1.02–1.32)
P_{trend}		0.001
Nitrite ug/1000 kcal		
Q1 (11.9)	457	1.0
Q2 (33.7)	488	0.99 (0.87–1.12)
Q3 (59.7)	554	1.07 (0.94–1.21)
Q4 (99.9)	603	1.12 (0.98–1.27)
Q5 (194.1)	617	1.11 (0.97–1.25)
P_{trend}		0.055

- **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_{trend}=0.001)
  - Nitrite (P_{trend}=0.055)
- **Iron**
  - Dietary intake (P_{trend}<0.001)
  - Heme Iron (P_{trend}=0.022)

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

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”
  - Proscuitto
- **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:**

  NO_2 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: $C_{12}H_{22}O_{11} + H_2O \rightarrow 4\ CH_3CHOHCOOH$

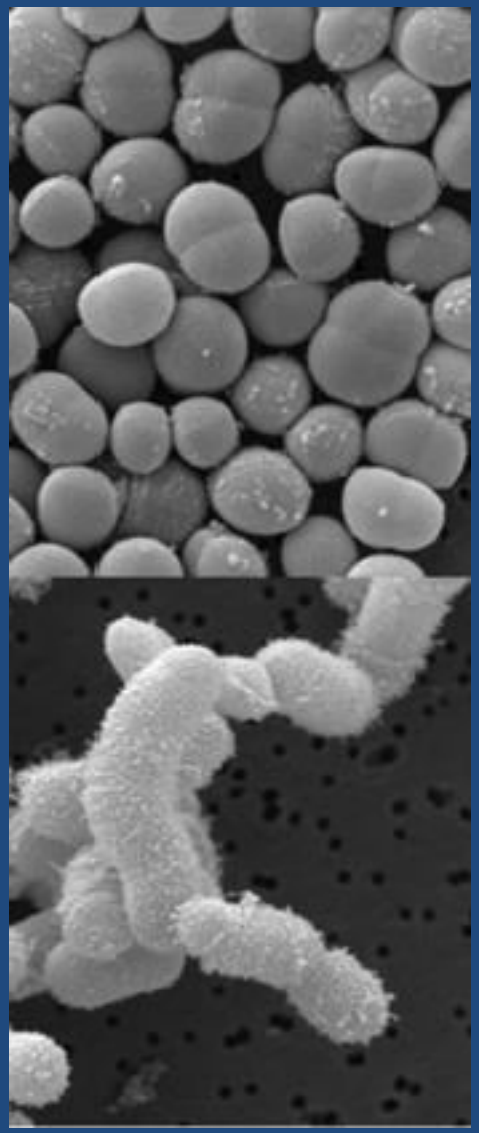
Heterolactic acid

- Glucose: $C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2$

Alkaline (*Bacillus*)

- Proteins → amino acids + NH_3

Nitrogen metabolism



Bacterial

Anaerobic

Dissimilatory Nitrate/Nitrite Reduction-

NO_3-> NO_2 (Nitrate reductase)

NO_2->NO (Nitrite reductase)

Ammonification

NO_2->NH_3->NH_4 assimilation

Eukaryotic

Aerobic

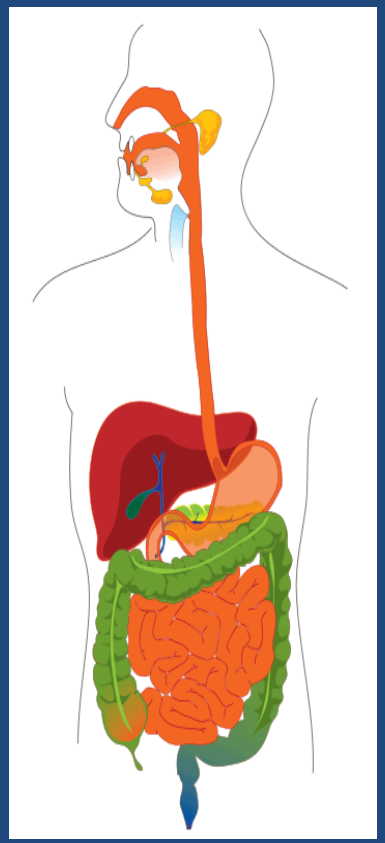
Arginine +O_2-> NO (Nitric Oxide Synthase)

Abiotic

NO_2->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

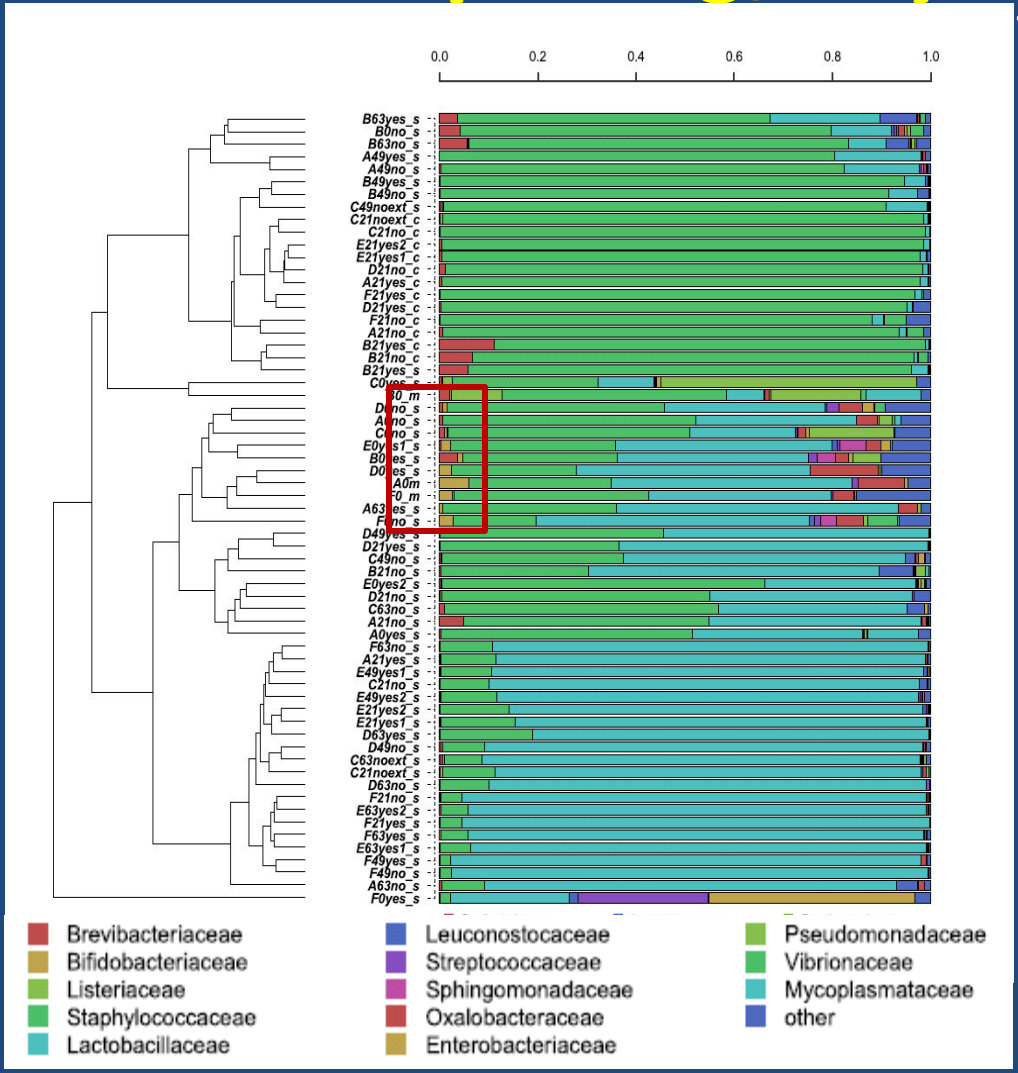
Gut:

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

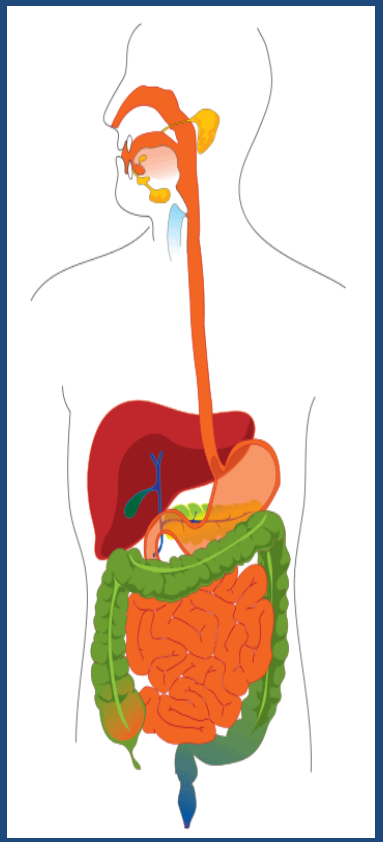
Physico-chemical changes
water potential is reduced
pH is reduced

NO can be formed

Polka et al 2015 Food Microb.46:342.

Microbial Mechanisms Associated with Dietary Intake of Fermented Meat





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Nitrate Reduction

Stomach:

Disproportionation and NOC formation

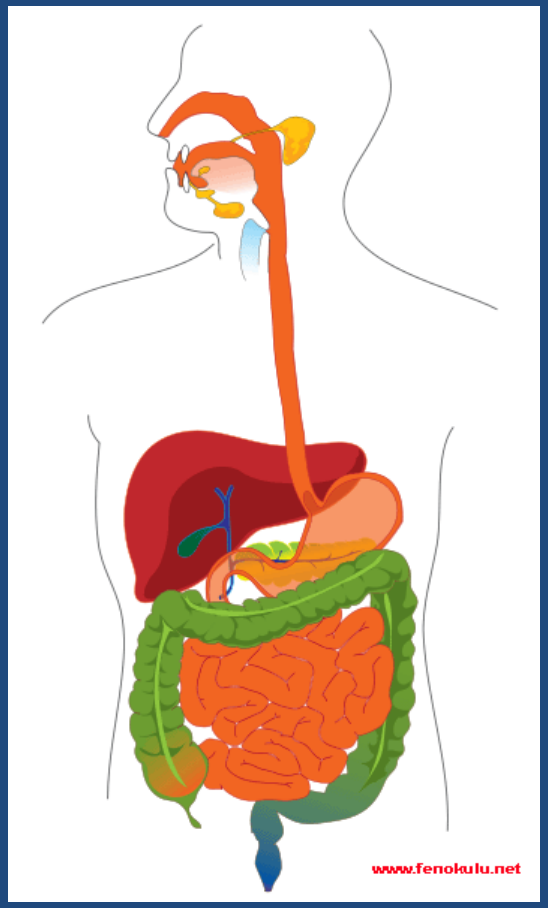
Gut:

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)

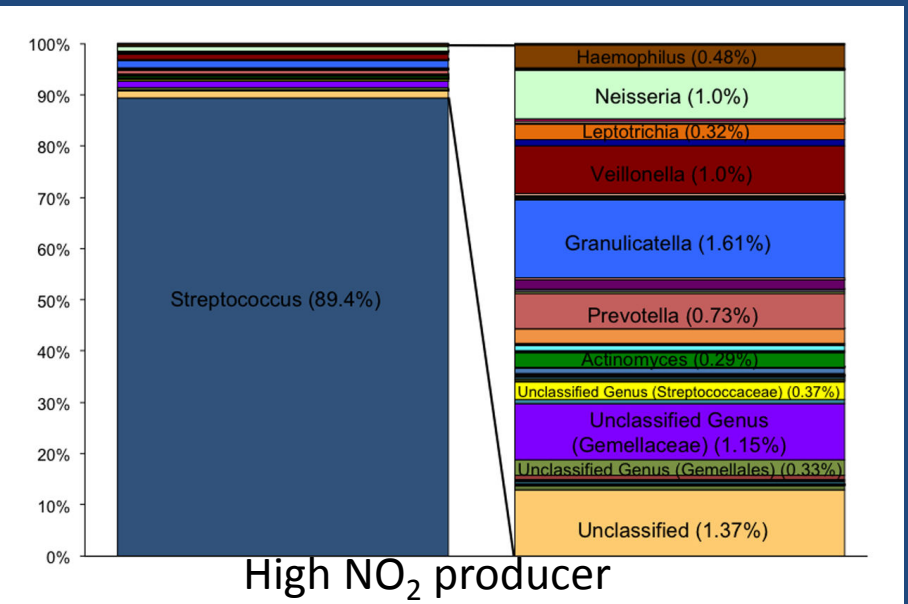
Ingested dietary NO_3

Enters blood stream in the small intestine

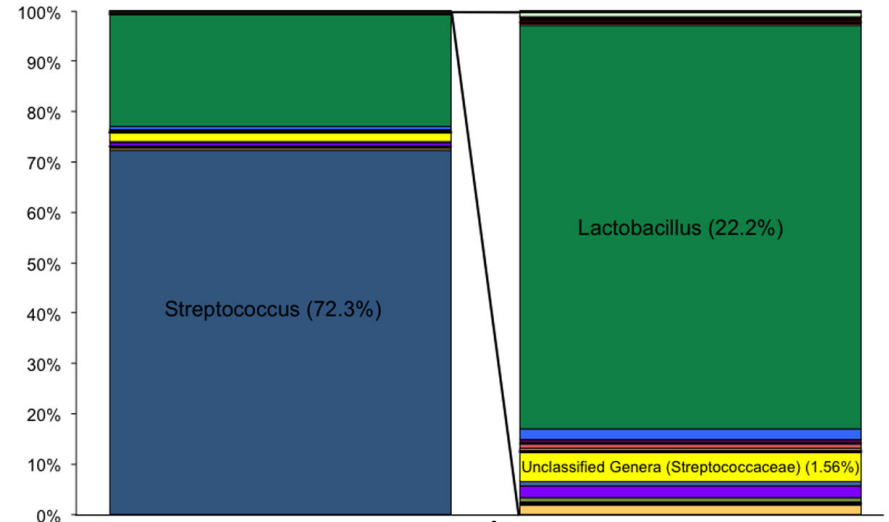
Combines with endogenous NO_3

Up to 25% actively taken up by salivary gland

Nitrate Reducing Bacteria in the Oral Cavity a source of NOC Precurors



High NO_2 producer



Low NO_2 producer

Nitric oxide synthase independent/O_2 independent pathway (Microbial)

Oral bacteria->NO_3->NO_2

Tongue microbiome from High/Low NO_2 producers

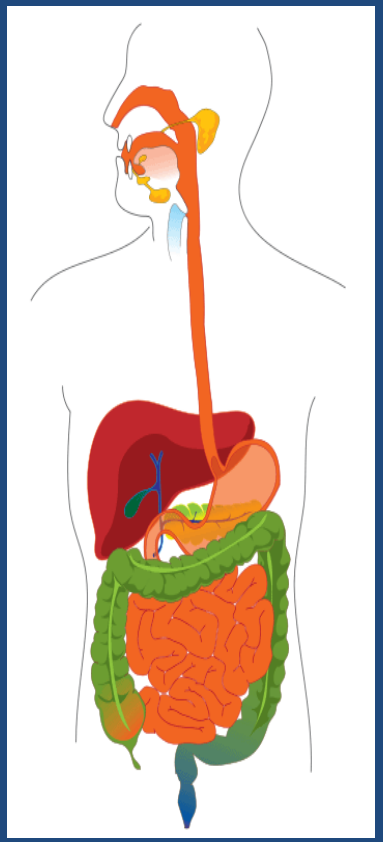
High producers more diverse

Functional genes enriched for nitrate reduction

(Hyde, E. et al.; 2014;3:e88645)

Microbial Mechanisms Associated with Dietary Intake of Fermented Meat





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Disproportionation and NOC formation

Gut:

NO and NOC Formation

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Nitrosation in the Stomach
Disproportionation and NOC formation

pH<5 NO_2 disproportionates to Nitric oxide (NO)

↓

Dietary proteins are decomposed to amines and amides

↓

Form N-nitroso compounds

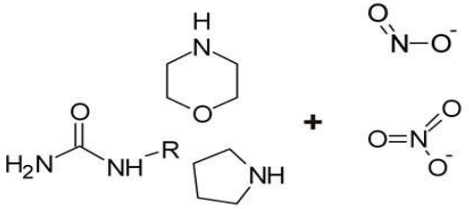
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N-nitroso compounds enter the gut and hydroxylated by CYP450

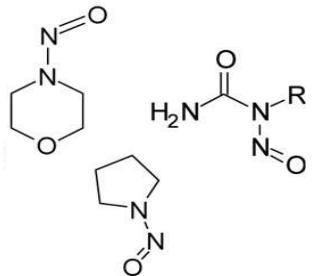
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Alkylating agents that form DNA adducts that cause mismatch and miscoding

Amine/Amide + NO

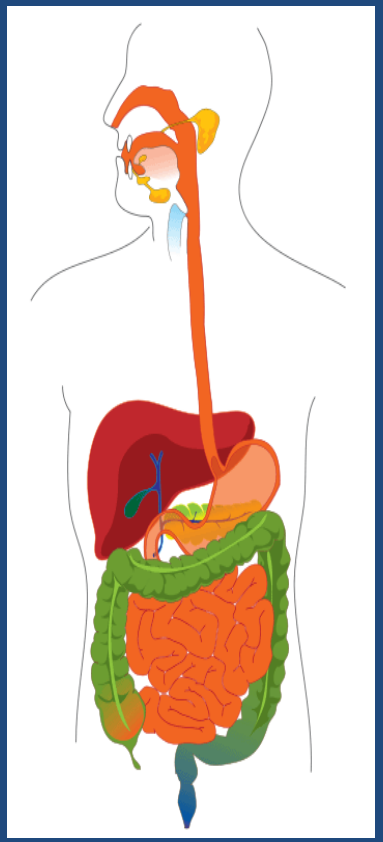


N-nitroso compounds



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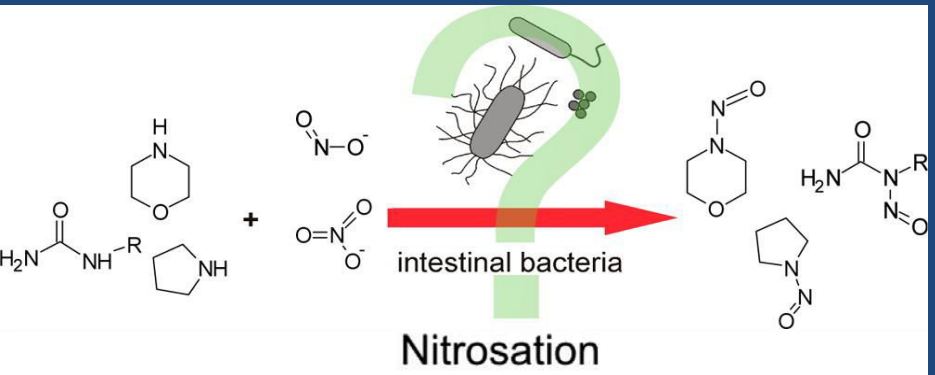
Gut:

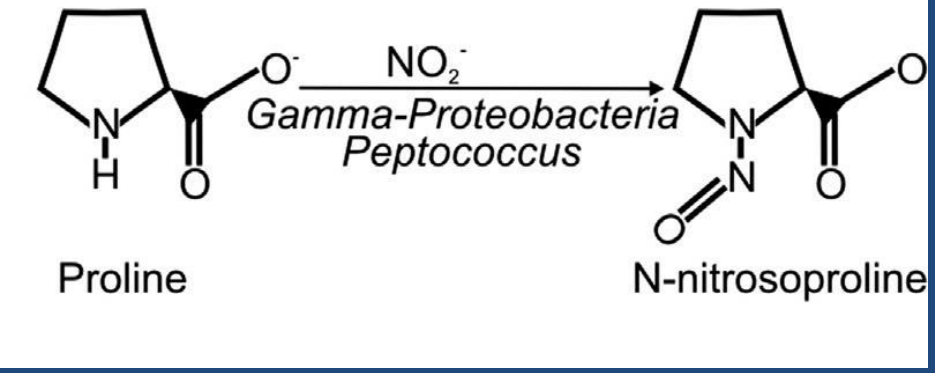
NO and NOC Formation

Protein degradation

Lipoperoxidation/Hemin

Microbial Nitrosation in the Gut





30% of NO_3 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 NO_3 in drinking water

(Massey 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

We Are What We Eat: Mammals and Their Gut Microbes Cluster by Diet



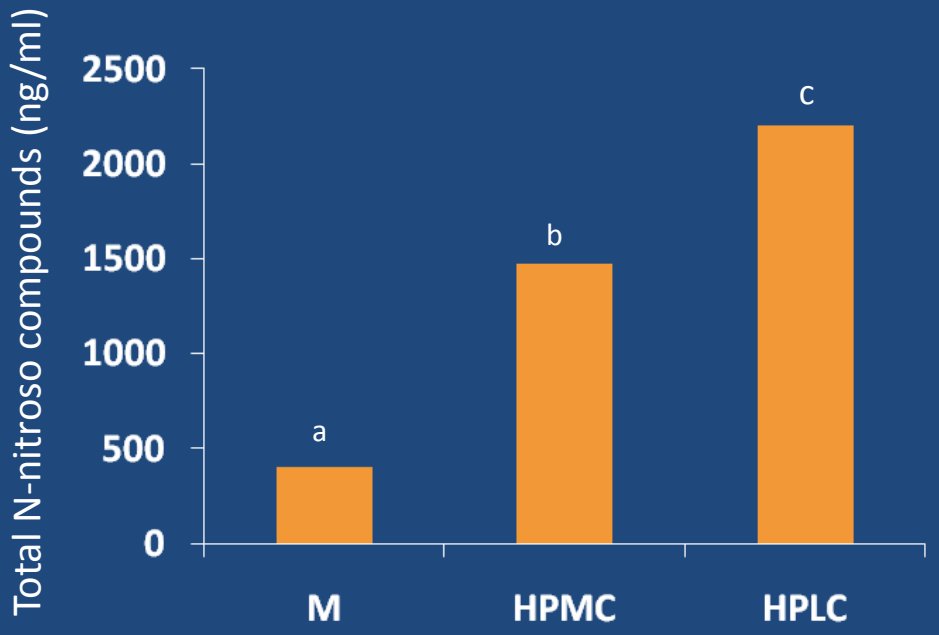
Herbivores

Omnivores

Carnivores

Ley, RE et al., Science, 2008, 320:1647

Gut Microbiome Affects Exposure: N-nitroso Compounds



Protein, %	13	28	29
Fat, %	37	37	66
Carb, %	50	35	5
NSP, g	22	9	13

a,b,c significantly different $p<0.001$

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

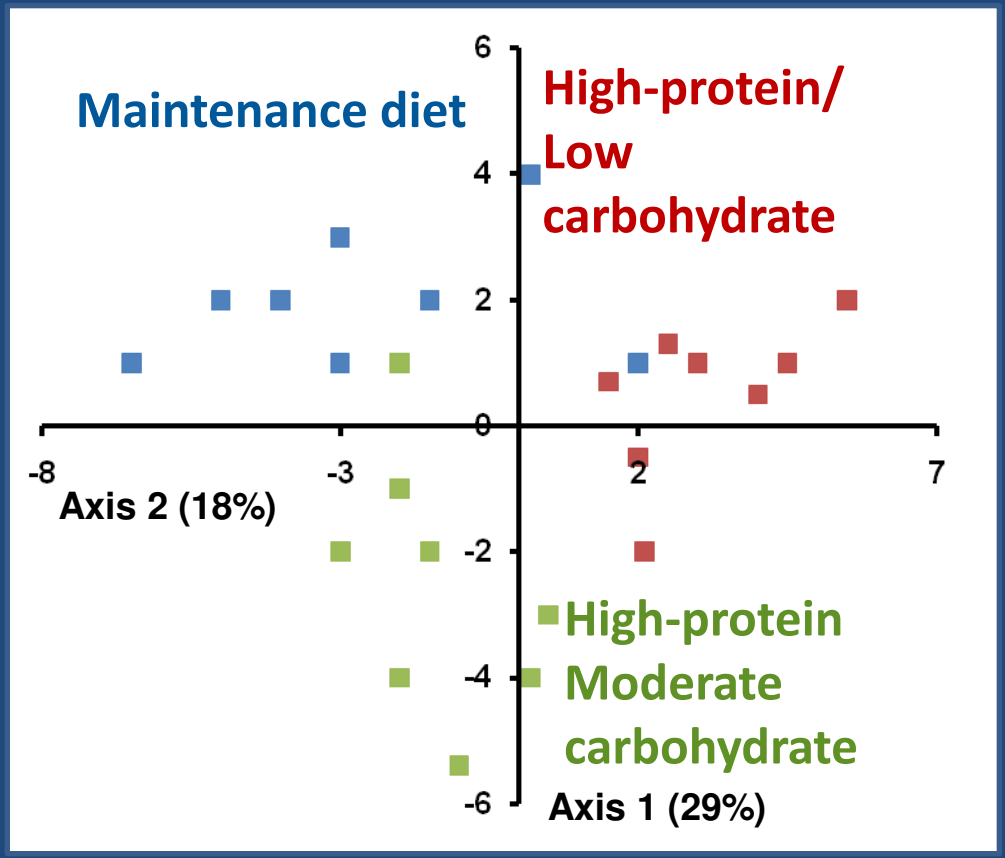
HPMC high-protein, medium carbohydrate

HPLC high-protein, low carbohydrate

- > N-nitroso compounds

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

Gut Microbiome Affects Exposure: N-nitroso Compounds



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

  HPLC diet :

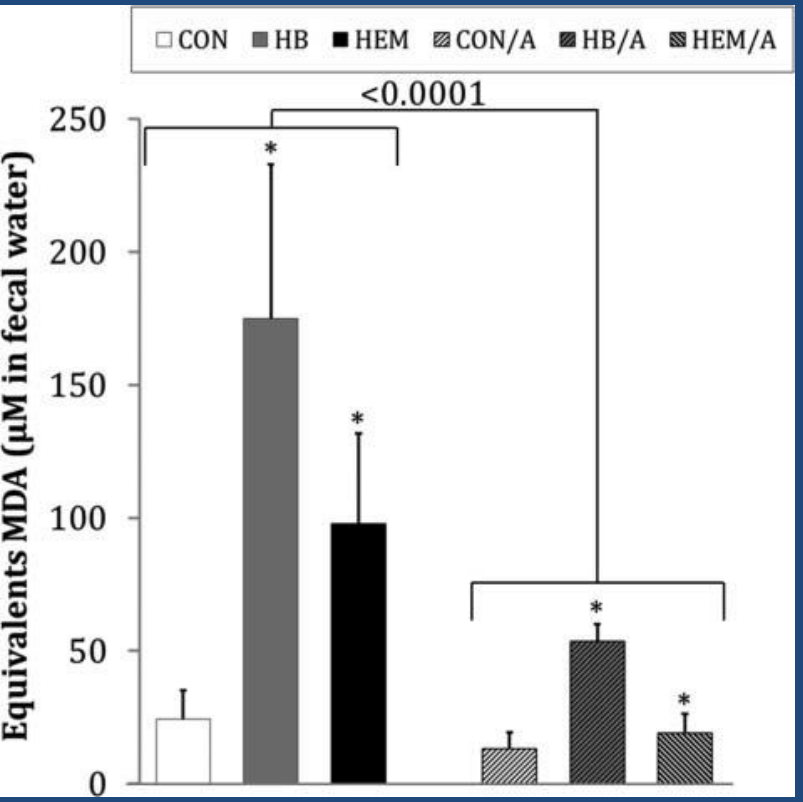
  >N-nitroso compounds

  < Butyrate

  <*Roseburia/ Eubacterium rectale*

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

Heme Iron, Lipoperoxidation, and the Microbiome



- Heme-induced lipoperoxidation forms Malondialdehye (MDA)-> DNA adducts genotoxic
- Rats given Hemoglobin or Hemin
- Antibiotics reduced lipoperoxidation

  Martin et al, 2015, Nutr.Cancer,67:119
- Change in the microbiome?

  + Catalase; $-H_2O_2$; lipoperoxidation -

  Bifidobacteria, Lactobacillus, and Streptococcus

  Zhang et al 2013,World. J. Microbio.29:12

  - Catalase; $+H_2O_2$; 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 ?

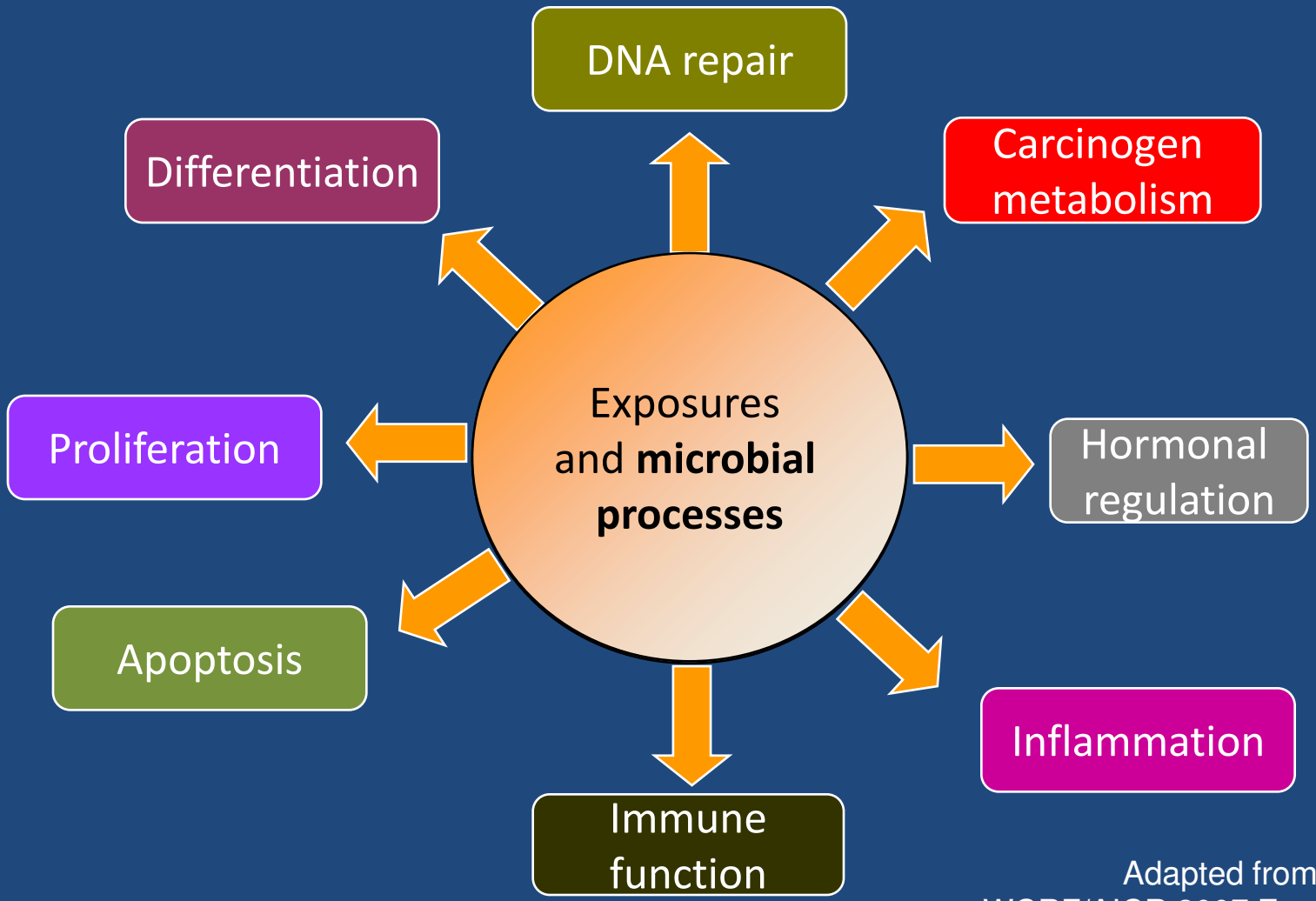


Supported by: US National Cancer Institute
FHCRC

FRED HUTCHINSON CANCER RESEARCH CENTER

Exposures and Cellular Processes Linked to Cancer

Involvement of Microbial Processes



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)

(Alexander DD et al, 2010, Eur J Cancer Prev., 19:328)

Colorectal adenomas

5 prospective studies (Highest vs Lowest SRR= 1.17)

(Xu, X. et al. 2013. Int J Cancer 132:437)

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