Diet and Cancer
with Special Reference to Colon Cancer

Stephen J D O’Keefe MD, MSc, FRCP
Division of Gastroenterology & Nutrition
Department of Medicine
University of Pittsburgh, PA
The Power of Epidemiology

- Increase Risk
  - Red meat*
  - Animal fat
  - Processed* meats
  - Obesity
  - Inactivity
  - Alcohol
  - Cigarettes

- Decrease Risk
  - Fiber*
  - Vegetables
  - Calcium
  - Fish oils
  - Antioxidants, selenium
  - Folic acid
  - Postmenopausal hormones

Colon cancer rates per country

* Evidence “convincing” 2010 Continuous Update Report from the World Cancer Research Fund review and meta-analysis of 43 cohort or randomized controlled trials

Approximately 90% of GI cancers are due to differences in diet

Fiber

• Complex carbohydrate in diet from plant sources that escapes small bowel digestion and thus reaches the colon: food for the colonic microbiota

• First attributed to low colon cancer prevalence in Africans by Burkitt

• EPIC study estimated 40% risk reduction in populations with low dietary fiber that doubled their fiber intake (*Bingham et al. Lancet 2003 361:1496*)
Red Meat & Fat

• Red Meat:
  – increase luminal carcinogens through heme, microbial metabolism of protein residues (nitrosamines, PAHs), and ‘barbequing’ – heterocyclic amines

  There was a 35% increase in CRC risk when more than 160 g/d of red and processed meat intake was compared with less than 20 g/d. In addition, the increase in CRC risk associated with high red and processed meat was higher in the group with low fibre intake than in the group with high fibre (EPIC 2010)

• Fat:
  – induces hepatic synthesis of bile acids; colonic microbes convert BA to $2^y$ BA which are carcinogenic
  – Taurine bile acids induce a blossom of *Bilophila wadsworthia* which produce H$_2$S which is genotoxic
  – Saturated fat has inflammatory actions mediated through prostanoid metabolism
Meat and Vegetables

• Spinach reduces the proliferative effect of heme in rat colonic mucosa
  – *De Vogel, et al. Carcinogenesis 2005*

• In humans, resistant starch reduces the proliferative and oncogenic effects of a high meat diet
  – 300g meat/day for 4 weeks, with/without 40g resistant starch, supplement prevented increase in oncogenic miRNA observed with red meat (300g/d) alone. *Humphries et al, Cancer Prev Res 2014*
Fruit and Vegetables

• Suppress colon cancer due antioxidant and antineoplastic properties due to their contents of vitamins (vitamin C, folate), micronutrients (selenium, calcium), and bioactive phytochemical compounds, such as phenolics, generally categorized as phenolic acids, flavonoids, stilbenes, coumarins, and tannins

  – all released by microbial degradation of cell walls and activation
Colon Cancer is a Westernized Disease
- It takes 1 generation to change

Le Marchand. Journal of the National Cancer Institute Monographs No. 26, 1999
Westernization

- Westernization has been associated with dramatic increases in expected lifespan from less than 25 before the Industrial Revolution to over 80 years today.
- This change is too rapid to be attributed to genetic evolution.
- But it can to the remarkable ability of humans to adapt to their environment.
- Ability to avoid life-threatening events such as perinatal complications, acute infections, trauma and war.

*Emergence of “westernized diseases”, which include colon cancer: present the most serious threat to public health in the USA today.*
Food

• From what we know of the process of evolution, dietary needs of every organism are genetically determined, and some have argued that our health will be better maintained by the diet that first established *Homo Sapiens* in the Paleolithic Era in Africa, a period that lasted from about 2.5 million years ago to 11,000 years ago.

• Coprolite analysis from cave dwellers hundreds of thousands of years ago show evidence of high grain consumption.
Evidence for dietary change but not landscape use in South African early hominins

Vincent Balter\textsuperscript{1}, José Braga\textsuperscript{2}, Philippe Télouk\textsuperscript{1} & J. Francis Thackeray\textsuperscript{3}

- Recent advances in dental microwear and stable isotope technology
- composition of foods consumed from by early hominins (\textit{Australopithecus}) and our \textit{Homo} ancestors who evolved in eastern Africa around \textbf{4 million years ago}
- dietary consumption was diverse, from \textit{Paranthropus robustus} consuming chimpanzee-like fleshy fruits and soft, young leaves (browsers) to \textit{P. boisei} living on a diet rich in tropical grasses and some sedges (grazers)

- a diet that is indistinguishable from that consumed by warthogs, hippopotami and zebras!
The Diets of Early Hominins

Peter S. Ungar\textsuperscript{1*} and Matt Sponheimer\textsuperscript{2*}

Diet changes are considered key events in human evolution. Most studies of early hominin diets focused on tooth size, shape, and craniomandibular morphology, as well as stone tools and butchered animal bones. However, in recent years, dental microwear and stable isotope analyses have hinted at unexpected diversity and complexity in early hominin diets. Some traditional ideas have held; others, such as an increasing reliance on hard-object feeding and a dichotomy between \textit{Australopithecus} and \textit{Paranthropus}, have been challenged. The first known evidence of C\textsubscript{4} plant (tropical grasses and sedges) and hard-object (e.g., seeds and nuts) consumption dates to millions of years after the appearance of the earliest probable hominins, and there are no consistent trends in diet change among these species through time.

\textsuperscript{1*}Department of Anthropology, Texas A&M University, College Station, Texas 77843-3126, USA.

\textsuperscript{2*}Department of Anthropology, University of California, Los Angeles, California 90095-1568, USA.
The Western Diet

- Grilled or fried meat
- Fat
- Carbohydrate
- Alcohol
- Preservatives
- Fruit and vegies??
- Fiber????????????
Hypothesis

It is not the Diet

It is the Microbiome-Metabolome
BUT IT’S FAR MORE COMPLICATED THAN THAT!
Microbial Products: the “Good” and the “Bad”

- Short chain fatty acids: acetate, propionate, butyrate
- Hydrogen, methane, hydrogen sulfide
- Secondary bile acids
- Vitamins
- Polyphenols
- Toxins, inflammatory agents
- Anti-inflammatory substances

So final risk is determined by BALANCE
Saccharolytic Fermentation

\[ 59 \text{C}_6\text{H}_{12}\text{O}_6 + 38 \text{H}_2\text{O} \rightarrow 60 \text{CH}_3\text{COOH}(\text{acetate}) + 22 \text{CH}_3\text{CH}_2\text{COOH} (\text{propionate}) + 18 \text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} (\text{butyrate}) + 96 \text{CO}_2 + 256 \text{H}^+ \]
**FOOD**

**GI TRACT**

**DIGESTION**

**ABSORPTION**

**BODY**

**LIVER PANCREAS**

**DIGESTION**

**ABSORPTION**

**INDIGESTIBLE RESIDUES**

**BILE ACIDS**

**PROTEIN**

**FIBER, PLANT CELL WALLS, GLYCANS**

**Deconjugation**

**Protein degradation, Fermentation**

**Degradation, Synthesis, Fermentation**

**2β bile acids**

**NH₃, phenolics, aromatics, H₂S, choline**

**Phytochemicals: phenolics, antioxidants**

**Vitamins: folate, biotin, niacin, B12**

**Short chain fatty acids:**

**Butyrate, acetate, propionate, CH₄**

**Inflammatory and Carcinogenic**

**Ammonia:** barrier function, mucus, mucosal permeability proliferation

**Branch chain fatty acids:** inflammatory

**Aromatic amino acids:** phenolics, indoles, p-cresol, N-nitrosoamines

**Hydrogen sulfide:** inflammatory, DNA damage, genotoxic

**Cell metabolism:** energy supply

**Genetic-Epigenetic regulation:** histone deacetylase inhibition, miRNA, down-regulation of canonical Wnt-signaling

**Anti-proliferative:** p53, p21 activation, reduced cell cycling, apoptosis

**Immunomodulatory and anti-inflammatory:**

GPR43, GPR109α activation, T(reg) activation of Foxp3 and IL-10 expression, NF-κB suppression

**Mucosal health and defence:** mucin synthesis, tight junctions, trefoil factors, antimicrobial peptides, heat shock proteins, transglutamase, β-glucuronidase activity

**Microbiota homeostasis:** phenolics, antioxidants

**CANCER RISK**
Current studies and clinical trials strongly suggest that HDAC inhibitors such as trichostatin A and sub-eroylanilide hydroxamic acid, which like butyrate, induce p21Waf1/Cip1 expression, are effective in arresting cancer cell proliferation and lead to differentiation (as in acute promyelocytic anemia) or apoptosis.
Utilized gnotobiotic mouse models colonized with wild-type or mutant strains of a butyrate-producing bacterium to demonstrate that fiber does have a potent tumor-suppressive effect but in a microbiota- and butyrate-dependent manner.
The Synthesis of Vitamins by the Colonic Microbiota

O’Keefe et al J Nutr 2009

![Graph showing the synthesis of vitamins by the colonic microbiota. The x-axis represents Folate, Biotin x10, B12, and Thiamin, while the y-axis represents units: Folate, Biotin and thiamin: mg/d (mg), B12 ug/d (ug).]

Dietary
Colonic Evacuates

units: Folate, Biotin and thiamin: mg/d (mg), B12 ug/d (ug)
Addition of 0.2% deoxycholic acid for 8–10 months to the diet of 18 wild-type mice induced colonic tumors in 17, and cancer in 10. Addition of the antioxidant chlorogenic acid at 0.007% to the DOC-supplemented diet significantly reduced tumor formation.
Figure 2. Dissimilatory metabolism of $N$-containing substrates in the large gut, and the physiologic significance of the end products of amino acid fermentation.
Pathway: CoA-transferase
Species: Eubacterium rectale
         Roseburia spp.
         Coprococcus catus
         Faecalibacterium prausnitzii
         Anaerostipes spp.
         Eubacterium hallii

Butyrate kinase
Species: Coprococcus eutactus
         Coprococcus comes

Intestinimonas AF211, Fusobacterium nucleatum
African-African American Studies

• Africans rarely get colon polyps or cancer <5:100,000

• African Americans have the highest prevalence of colon cancer in the USA: >65:100,000

Protocol

• A series of studies on groups of 12-20 normal healthy subjects from each population, aged 50-65

• Compared differences in:
  – Diet
  – Colonoscopy
  – Fecal and colonic microbiota
  – Fecal and colonic microbial metabolites
  – Mucosal inflammation (CD3+, CD68+) and proliferation (Ki67) as biomarkers of cancer risk
Epithelial Proliferation as a Biomarker of Colon Cancer Risk

Colonic Epithelial Proliferation Rates
Split By: groups
Error Bars: ± 1 Standard Error(s)

O’Keefe et al. Am J Gastroenterology 1999
Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans$^{1-4}$


Am J Clin Nutr 2013
Targeted Analysis

• Africans had higher levels of fecal and colonic microbial butyrate producers (qPCR) and butyrate levels (GC)

• African Americans had higher levels of secondary bile acid synthesizing bacteria (qPCR) and secondary bile acids (LC-MS)
But there are other environmental differences than might provide the explanation

e.g.
• Food storage, preservation, and preparation
• Sanitation
• Housing
• Electricity
• Transport
• Physical activity
ARTICLE

Received 23 May 2014 | Accepted 20 Jan 2015 | Published 28 Apr 2015

DOI: 10.1038/ncomms7342

Fat, fibre and cancer risk in African Americans and rural Africans

Stephen J.D. O’Keefe¹, Jia V. Li², Leo Lahti³,⁴, Junhai Ou¹, Franck Carbonero⁵,†, Khaled Mohammed¹, Joram M. Posma², James Kinross², Elaine Wahl¹, Elizabeth Ruder⁶, Kishore Vipperla¹, Vasudevan Naidoo⁷, Lungile Mtshali⁷, Sebastian Tims³, Philippe G.B. Puylaert³, James DeLany⁸, Alyssa Krasinskas⁹, Ann C. Benefiel⁵, Hatem O. Kaseb¹, Keith Newton⁷, Jeremy K. Nicholson², Willem M. de Vos³,⁴,¹⁰, H. Rex Gaskins⁵ & Erwin G. Zoetendal³
You can change your biomarkers of colon cancer risk within 2 weeks of change to an African or western diet.
Age-Specific Cancer Incidence Rates, 1994-2008
Alaska Native and US White Populations

Colon and Rectum, Men and Women Combined

Rate per 100,000 population

Age

AK Native

US White
Summary

• The microbiota behaves as a community wherein intermicrobial interaction strives to produce a metabolic phenotype that supports colonic health and function.

• It has a genetically determined need for food residues derived from a healthy balanced diet.

• Provision of an imbalanced diet leads to disturbance in structure and function, with unopposed production of metabolites that can induce inflammation and proliferation which increase risk of neoplasia.
Conclusions

• From epidemiological studies, we know that westernization of the diet leads to an increase in colon cancer within one generation

• Our results show that a change in diet composition produces immediate effects on the metabolic phenotype of the colonic contents associated with reciprocal mucosal biomarkers of cancer risk

• Our results suggest that current guidelines for the consumption of fiber rich foods are too low and increasing the fiber to >50g/d and reducing fat by half in African Americans, and indeed in all populations consuming a western diet, is likely to have an immediate effect on colon cancer risk

• They warn against westernization of the African diet, where reduction in fiber intake will lead to suppression of butyrogenesis and a rapid progression of chronic colonic inflammation to cancer
Acknowledgements

O’Keefe Lab:
• Junhai Ou, Al Cecchetti, Khaled Mohammed, Liz Ruder, Kishore Vipperla, Hatem Osama,
• Jim DeLany (Endocrine), Alyssa Krasinskas (Pathology), Priya Iyer (UPCI), Elaine Wahl (CTRC)

South African Studies:
• Prof Tian van der Merwe, MEDUNSA, Prof Keith Newton, Vasudevan Naidoo, Lungile Mtshali UKZN

Nicholson Lab: Imperial College, London: The Metabolome
• Jeremy Nicholson, James Kinross, Jia Li

deVoss Lab: Microbiology, Wageningen University, The Netherlands
• Erwin Zoetendal, Leo Lahti

Gaskins Lab: University of Illinois at Urbana: Genomic Biology
• Rex Gaskins, Gerardo Nava, Franck Carbonera

FUNDING

O’Keefe: NIH: R01 CA135379

Nicholson: National Institute for Health Research (NIHR) Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London, the Academy of Medical Sciences who funded part of the metabolic profiling work.

deVoss: The Spinoza Award of the Netherlands Organization for Scientific Research, the ERC Advanced Grant 250172 (Microbes Inside) of the European Research Council and the Academy of Finland (Grant 141140).