Pilot Study to Analyze the Colonic Microbiome and Metabolome that may Increase the Risk of Colon Cancer in Alaska Native people

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Diet and Colon Cancer

- Colon cancer is the second leading cause of cancer deaths worldwide.
- There is overwhelming evidence based on worldwide epidemiological studies and experimental studies that diet drives colon cancer risk.
DIET → COLON CANCER

Colon cancer rates per country

<table>
<thead>
<tr>
<th>Increase Risk</th>
<th>Decrease Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Red meat</em></td>
<td><em>Fiber</em></td>
</tr>
<tr>
<td><em>Processed meats</em></td>
<td>Vegetables</td>
</tr>
<tr>
<td>Animal fat</td>
<td>Fiber-rich Foods*</td>
</tr>
<tr>
<td>Obesity</td>
<td>Calcium</td>
</tr>
<tr>
<td>Inactivity</td>
<td>Fish oils</td>
</tr>
<tr>
<td>Alcohol</td>
<td>Antioxidants, Selenium</td>
</tr>
<tr>
<td>Cigarettes</td>
<td>Folic acid</td>
</tr>
<tr>
<td>Postmenopausal hormones</td>
<td></td>
</tr>
</tbody>
</table>

* Evidence “convincing” from 2010 World Cancer Research Fund review and meta-analysis of 43 controlled trials
Colon Cancer is a Westernized Disease

- It takes 1 generation to change

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

It is not the Diet

It is the Microbiome-Metabolome
Humphreys et al. conducted randomized controlled cross-over study in healthy volunteers given either a high red meat diet (300g/d) or a high red meat diet plus resistant starch supplement for 4 weeks.

The high red meat diet increased rectal mucosal oncogenic miRNA (miR17–92 cluster) expression and cell proliferation, in association with a decrease in miR17–92 target gene transcript levels.

Importantly, all these effects were negated by increasing butyrogenesis through fiber supplementation (40g of butyrylated high amylose maize starch per day, of which 60% is resistant).

Humphreys et al. Cancer Prevention Research Research 2015
The Study of Low, High and Extreme Cancer Risk Populations

- 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
- Alaska Native People have the highest recorded incidence in the world at >100:100,000

Colon Cancer Risk is Uniformly High

<table>
<thead>
<tr>
<th>Tribal Health Region</th>
<th>Rates per 100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Alaska</td>
<td>92</td>
</tr>
<tr>
<td>Aleutians</td>
<td>93</td>
</tr>
<tr>
<td>Anchorage</td>
<td>80</td>
</tr>
<tr>
<td>Arctic Slope</td>
<td>179</td>
</tr>
<tr>
<td>Bristol Bay</td>
<td>93</td>
</tr>
<tr>
<td>Copper River/PWS</td>
<td>143</td>
</tr>
<tr>
<td>Interior</td>
<td>103</td>
</tr>
<tr>
<td>Yukon-Kushokwim</td>
<td>102</td>
</tr>
</tbody>
</table>

Dietary Intakes in Alaska Native People

- Fiber intake 11-13g/d: 2471 Kcal, Prot 112g/d, carbs 275g/d: Johnson J et al. *Int J Circumpolar Health* 2009;68(2):109-122

- 83% of Native adults in rural Alaska do not eat 5 servings of fruits and vegetables per day
  - *Behavioral Risk Factor Surveillance Survey, State of Alaska Department of Health and Social Services, 2006*

- Recommendations to continue use of traditional foods and increase intake of fruits and vegetables are consistent with local attitudes. Our findings indicate that increasing the availability of fruits and vegetables would be well received. *Johnson 2011*

- Bersamin et al noted that fiber intake was 12g/d with westernized foods, but only 5.6g/d with a traditional diet: *Int J CH 2007*
Fig 1: Missing Essential Components of the Alaskan Native Diet

- Fruits, vegetables, grains
- Phytochemicals
- Meat from terrestrial and marine animals, fish proteins and oils

Essential for Colonic Health

Essential for Body Health

High biological value proteins and fats
The African Diet

- First recognized by Dennis Burkitt to be associated with a low incidence of non-infective colonic diseases, and particularly with colon cancer
- The traditional diet was rich in complex carbohydrates from grains, beans, and wild green vegetables and fruits, and low in meat and fat
- Fiber content was >50g/d
  
  *Burkitt, D.P., Epidemiology of cancer of the colon and rectum. Cancer. 1971*

- Diet Today: 1/3 animal protein and fat, 3x fiber
  
  *O’Keefe et al J Nutr 2005*
But, is it the Diet?

African-American Diet Exchange
The Dietary Switch

<table>
<thead>
<tr>
<th></th>
<th>Fat</th>
<th>Fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native Africans g/d</td>
<td>145</td>
<td>7</td>
</tr>
<tr>
<td>African Americans g/d</td>
<td>41</td>
<td>55</td>
</tr>
</tbody>
</table>
You can change your biomarkers of colon cancer risk within 2 weeks of change to an African or western diet.
The Anchorage Pilot Study

- 20 adults undergoing screening colonoscopy
  - Pre-endoscopy fecal sample for microbiota and their metabolites
  - Dietary assessment
  - Blood sample for delta $^{15}$N and delta $^{13}$C isotope ratios to assess market/traditional food intakes
  - Subsample from Barrow
The Microbiome: MDS Plot

Rotated MDS: Centroids Labeled

Population:
- alaskan
- american
- african

df = 2
SS = 1.3403
MS = 0.6701
F = 7.9582
R^2 = 0.2056
p-value = 0.0001
eta^2 = 0.2056
Alaska Native people: Anchorage sample
Native Africans

Rural African sample
The Metabolome: H1NMR: OPLS-DA cross validated scores plot

African vs Alaskan
Metabolites labelled here have a p<0.05 after Benjamini-Hochberg correction.
Butyrate synthesis: \textit{bcoA}

Significant differences based on Mann-Whitney test or unpaired t-test (if data is normally distributed) with *=p<0.05; **=p<0.01; ***=p<0.001; ****=p<0.0001.

Butyrate synthesis: \textit{buk}

Significant differences based on Mann-Whitney test or unpaired t-test (if data is normally distributed) with *=p<0.05; **=p<0.01; ***=p<0.001; ****=p<0.0001.

Bile acid conversion: \textit{baiCD}

Significant differences based on Mann-Whitney test or unpaired t-test (if data is normally distributed) with *=p<0.05; **=p<0.01; ***=p<0.001; ****=p<0.0001.
Significant differences based on Mann-Whitney test or unpaired t-test (if data is normally distributed) with *=p<0.05; **=p<0.01; ***=p<0.001; ****=p<0.0001.

Acetate

Propionate

Butyrate
The FIRST Study

- Restore normal fiber content of the diet to 50g/d by supplementation with resistant starch fiber
- 4 week RCT to determine whether we can suppress biomarkers of colon cancer risk
Summary

- The pilot study provides supportive evidence that
  - Dietary fiber intake is low
  - Colonic fermentation is low
  - Butyrate production is low
  - Secondary bile acids are high
Conclusion

- There is overwhelming evidence that restoring the dietary intake of fiber-rich foods will reduce the extreme risk of getting and dying of colon cancer in Alaska Native people
PREVENTION IS BETTER THAN CURE

Figure 1. Relative comparison of 5-year disease-free survival stratified by adjuvant treatment regimen.

Peritoneal carcinomatosis median survival is only 2 years after aggressive therapy.
Cultural Rigor
FINAL REPORT ON
THE ALASKA TRADITIONAL DIET SURVEY

“Because eating native food is important,”
“Eating traditional food is my way of life,” and “[it is the] Eskimo way of life.”
Aquttuk