FOR DEBATE

Vitamin B12 and the colonic microflora

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University of Cranfield
The Microbe

The microbe is so very small
We cannot make him out at all
Though many sanguine people hope
To see him down a microscope....

H Belloc (1896)
Patient Histories

- Patient SC – female 32yrs
  Chronic fatigue and diarrhoea

- Patient JD – female 79yrs
  Chronic fatigue and diarrhoea

- Patient SR – male 37yrs
  Chronic fatigue & mouth ulcers

- B12 157
- B12 187
- B12 238
Patient Histories

- No evidence of
  - Pernicious Anaemia
  - Small bowel diverticula
  - Coeliac disease
  - Crohn’s disease
  - Chronic pancreatitis

In all 3 patients fatigue improved after 6 injections of 1mg vitamin B12
  – but the benefits rapidly wore off!
Patient Histories

- Other symptoms eg. Diarrhoea continued
- Fatigue returned rapidly after completion of B12 injection course
Patient histories

- Diarrhoea and oral ulceration in all improved on exclusion diets.
- Specific food intolerances discovered – mainly grains, dairy products and caffeine.
- B12 injections needed to be repeated at 2-4 weekly intervals to prevent return of chronic fatigue.

- Final diagnosis of gastrointestinal symptoms – **Irritable Bowel Syndrome**
Most Frequent Causes of IBS

- Anxiety and Aerophagia
- Overload and Overflow
- Colonic Malfermentation causing food intolerances
Food Intolerance in IBS

- NOT a food allergy!
- No increase in serum IgE
- RAST and skin tests unhelpful
- No danger of anaphylaxis
- Large food helpings (100g) trigger symptoms
- Antihistamines ineffective
# Mechanisms of Food Intolerance

<table>
<thead>
<tr>
<th>Genuine</th>
<th>Assumed</th>
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<tbody>
<tr>
<td>Food allergy</td>
<td>Food aversion</td>
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<tr>
<td><strong>Pharmacological effects</strong></td>
<td>Psychological factors eg. Anxiety</td>
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<td>Sugar malabsorption</td>
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<tr>
<td>Colonic malfermentation</td>
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Rectal prostaglandin level in response to food challenge

Lancet 1982
Relationship between prostaglandin level & wet faecal weight

Alun Jones et al
Lancet 1982 ii
1115-1117
Exclusion Diet

(J.Hum.Nutr.And Dietetics, 1995)

253 Patients
Advised

28 (11%) Unable to follow diet
200 (79%) Completed 2 weeks
25 (10%) Lost to follow up

100 (50%) Improved
100 (50%) No Improvement
Fermentation

- Following digestion and absorption in the small intestine, bacteria in the large intestine ferment remaining food molecules.
- Substrates are starches, fibre, some fat and protein.
- Products are SCFA’s and BCFA’s, CO$_2$, H$_2$, H$_2$S, phenols and indoles.
- IBS $\rightarrow$ abnormal fermentation $\rightarrow$ increased H$_2$ production.
6 IBS Patients

Standard diet

Exclusion Diet

2 weeks free diet

6 controls

Day 14-whole body calorimetry
Median rates of total gas production (hydrogen and methane) over 24hrs by 6 IBS patients on a standard diet.
Median rates of total gas production (hydrogen and methane) over 24hrs by 6 IBS patients on a standard diet after taking an antibiotic.
Median rates of total gas production (hydrogen and methane) over 24hrs by 6 IBS patients on a standard diet
Median rates of total gas production (hydrogen and methane) over 24hrs by 6 IBS patients on a no fibre diet
Urine Analysis - Metabolomics

- 3 ml urine + internal standard
- Liquid injection and SPME
- Area under the curve
- Overall comparison - PCA
- Both take into account internal standard and creatinine
# Results – Mann Whitney U-test for pre-treatment faeces

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<tr>
<th>Compound</th>
<th>Control vs. Pre CD</th>
<th>Control vs. Pre UC</th>
<th>Control vs. Pre IBS</th>
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<td>1-propanol</td>
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<td>Butanoic acid, methyl ester</td>
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* Shows where compound was statistically significant between pairs
Food intolerance

Specific foods

Undigested residues

Colonic fermentation

normal gut flora

abnormal gut flora

non-toxic metabolites

destroyed by host enzymes

Asymptomatic

IBS

toxic metabolites

not destroyed

The Microbe

His jointed tongue that lies beneath
A hundred rows of curious teeth,
His seven tufted tails with lots
Of lovely pink and purple spots
On each of which a pattern stands,
Composed of forty separate bands;
His eyebrows of a tender green;
All this has never yet been seen….

H Belloc
Colonic Bacterial Flora in IBS

- Only 60 - 70% of flora can be cultured and identified
- No specific pathogen yet found
- Damage by antibiotics or gastroenteritis

- Flora is unstable
- Reduced counts of Lactobacilli and Bifidobacter
- Overgrowth of facultative anaerobes – from $10^5$ up to $10^8$
Colonic fermentation

- Fermentation increased in IBS
- Due to increased numbers of oxygen loving gram-negative bacteria
Vitamin B12 deficiency

- Pointers from small bowel overgrowth
  - Bacteria in small bowel have high affinity for B12
  - B12 absorbed by bacteria is NOT released
  - Bacteria concerned are oxygen loving gram negatives – as in IBS

- Could bacterial overgrowth in large bowel increase demands for B12?
Effects of B12 on chronic fatigue
<table>
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<th>Before B12</th>
<th>After B12</th>
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<tr>
<td>Mean</td>
<td>78.473*</td>
<td>44.291*</td>
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<td>*p =0.0188</td>
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<tr>
<td>SD</td>
<td>40.319</td>
<td>43.981</td>
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<tr>
<td>SEM</td>
<td>12.157</td>
<td>13.261</td>
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<tr>
<td>N</td>
<td>11</td>
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Serum B12 concentrations before and after injection

- P<0.001
B12 and the colonic microflora

- BUT:-
  - NO correlation between symptom scores and B12 concentrations.
  - Some subjects symptomatic when B12 levels in serum higher than those found in healthy controls.
  - Why does a B12 shot improve fatigue even when B12 levels are relatively high?
  - Homocysteine and methylmalonic acid, pointers to tissue B12 deficiency, not increased in these subjects.
B12 and the colonic microflora—previous work

In Man

‘May an anomaly of the intestinal flora be a factor aggravating B12 deficiency in achlorhydric gastritis?’

B12 and the colonic microflora

- In rats

  - Methyl malonic acid and vitamin B12 excretion of rats consuming diets varying in cellulose and pectin. *J Nutr* (1977) **108** 640-647

  - Dietary pectin shortens the biologic half-life of vitamin B12 in rats by increasing fecal and urinary losses. *J Nutr* (1989) **119** 1121-7
B12 and the colonic microflora

How could we further investigate it?

- Use more accurate tests of B12 status
- Measure half-life of B12 in subjects complaining of early fatigue (if radio-isotopes available)
- Measure B12 loss in faeces
- Search for abnormal bacterial metabolites in patients complaining of early fatigue
- Examine effect of B12 repletion on metabolites.
Vitamin B12 and the colonic microflora

Conclusions:-

B12 deficiency may occur in IBS without other causes being apparent.

Some patients develop symptoms of fatigue well before next B12 injection due.

IBS is associated with bacterial overgrowth and increased rates of colonic fermentation.

Does this increased bacterial activity affect B12 requirements?
The Microbe

His eyebrows of a tender green;
All this has never yet been seen.
But Scientists, who ought to know,
Assure us that it must be so!
Oh let us never, never, doubt
What nobody is sure about!

Hilaire Belloc (1896)
Vitamin B12 deficiency

- Pointers from small bowel overgrowth
  - Bacteria in small bowel have high affinity for B12
  - B12 absorbed by bacteria is NOT released
  - Bacteria concerned are oxygen loving gram negatives – as in IBS

- Could bacterial overgrowth in large bowel increase demands for B12?
Change in bacterial activity on elemental diet: Phenol on breath

Changes of phenol in different diets

-0.1
0
0.1
0.2
0.3
0.4
0.5
Beforediet1
AfterModulen
After E028

Mean alveolar Gradient

-0.1
0
0.1
0.2
0.3
0.4
0.5
Before diet 1
After Modulen
After E028

Mean
• Constipation and Musculoskeletal similar  
• IBS-increased compound  
• Compound decreases with diet-bacterial origin  
• It decreases to healthy levels/almost