The ageing gut microbiota and the impact of prebiotics

Gemma Walton
Overview

- The transition of age
- Ageing and disease risk
- Ageing – the microbiota – from birth to old age
- Prebiotics – what can they do
- Cancer risk?
- Immunosenescence and inflammageing??
How are we ageing?

- Population pyramid EU-28, 2001 and 2013
- (Eurostat)
What does the future hold?

- Increased longevity
- Reduced fertility

(1) 2012 provisional 2080 projections (EUROPOP2012).
Source: Eurostat (online data codes: demo_agegroup and proj_13homs)
Ageing

• “Ageing is the single most important risk factor underpinning the major chronic diseases. As healthy life expectancy has not increased at the same rate as longevity, the prevalence of age-related disease continues to increase.” Newcastle University
Age associated illness

- Diabetes
- Cardiovascular disease
- Cancers
- Depression
- Neurodegenerative diseases
- Mobility
- Vision
- Arthritis
- Hypertension
Gut microbiota changes – coincidence?

- The gut microbiota
  - Birth
  - Breast v Formula
  - Adulthood
  - Ageing
The ageing microbiota

- Bifidobacterial decline?
  - Mitsuoka, 1992; Hopkins et al., 2002; Woodmansey et al., 2004; Mueller et al., 2006
- Culturing

- Reduced bifidobacteria diversity (Zwielehner et al., 2009)
- Centenarian bifidobacterial reductions (Biagi et al., 2010)
The ageing microbiota

Other observations

- Bacteroides – higher proportion
- More *Clostridium* cluster IV
- Less *Clostridium* cluster XIV
- Claesson *et al.*, 2009
Gut changes

- A change in balance
- More proteolysis
- Less SCFA

- Colonic protein degradation – cancer links
Ageing

- Is there a magic bullet to help healthy ageing?
**In vivo**

A randomised crossover study investigating the effects of galacto-oligosaccharides on the faecal microbiota in men and women over 50 years of age

Gemma E. Walton\textsuperscript{a1} \textsuperscript{c1}, Ellen G. H. M. van den Heuvel\textsuperscript{a2}, Marit H. W. Kosters\textsuperscript{a2}, Robert A. Rastall\textsuperscript{a1}, Kieran M. Tuohy\textsuperscript{a1} and Glenn R. Gibson\textsuperscript{a1}

Not an elderly population
Target before changes occur
Study design

Samples 1-6 taken for:
Bacterial characterisation; Genotoxity; \textit{In vitro} SCFA
Study

39 volunteers took part in the study (18m, 21f)
Over the age of 50
Interval Plot of Bifidobacteria
Bars are One Standard Error from the Mean

<table>
<thead>
<tr>
<th></th>
<th>placebo</th>
<th>prebiotic</th>
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<tbody>
<tr>
<td>Bifidobacteria</td>
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<td></td>
</tr>
<tr>
<td>8.4</td>
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<tr>
<td>8.5</td>
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<td>8.6</td>
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<td>8.7</td>
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<td>8.8</td>
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<td>8.9</td>
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<td>9.0</td>
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<td>9.1</td>
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<tr>
<td>9.2</td>
<td></td>
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<tr>
<td>9.3</td>
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</tbody>
</table>

Panel variable: Treatment

* - p = 0.006, t-test, p = 0.014 – Friedman
Results

- Faecal water screening – 45% volunteers elevated levels of genotoxicity (i.e. $>30\%$)
Genotoxicity before and after prebiotic

DNA tail intensity (%) vs Volunteer number

- Vol. 19, 23, 28, 29, 30

- Red = before prebiotic
- Gray = prebiotic

Bars represent the percentage DNA tail intensity for each volunteer before and after prebiotic treatment.
Parallel Gut models

- 3 volunteers stool samples were used in the GM system
- Dosed with 2g GOS twice daily
Gut model

- Three stage continuous culture system
- pH and volume
- RT
- Faecal inoculum
- Steady state
Gut models - Vessel 3

Log10 cfu/ml

- Total bacteria
- Bifidobacteria
- Lactobacillus
- E. coli
- Bacteroides

Log10 Bacterial numbers / ml

- E. rectale
- C. coccoides
- C. histolyticum
- C. perfringes

= Steady state 1
= Steady state 2
SCFA in Vessel 1

SCFA in Vessel 2

SCFA in Vessel 3

SCFA concentration (mM)

Acetate
Propionate
Iso butyrate
Butyrate
Iso valerate
Valerate
Caproic

Acetate
Propionate
Iso butyrate
Butyrate
Iso valerate
Valerate
Caproic

SS1
SS2
Conclusions

• Bifidogenic effects seen in the GM and trial
• Increase in butyrate seen *in vitro*
• No genotoxic changes

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Re-addressing the balance

- Impact on health??

- Immunosenescence

- Loosely defined as decline in immune system brought on by ageing

- However - immune system is complex and multifaceted
Immunosenescence signs

- Decline of T-cell function
- Reduced progenitor B-cells
- Reduce natural killer cells
- Lower anti-body titre (humoural response)
- Inflammageing
How can prebiotics aid immune function?

- Increasing growth of beneficial bacteria thus inhibiting pathogenic growth
- Reducing stimulation of pro-inflammatory cytokines which cause chronic inflammation
Study design

10 week intervention
Blood and faeces collected
Bacterial populations results summary

Changes in faecal bacterial populations assessed through fluorescent *in situ* hybridisation (FISH)

- *Bifidobacterium spp.*
- *Bacteroides spp.*
- *Eubacterium rectale – Clostridium coccoides* (EREC)

were significantly higher after B-GOS compared to placebo
Natural Killer cell activity was significantly higher following B-GOS compared to placebo.

![Graph showing natural killer cell activity over time points (Baseline, wk 5, wk 10) for placebo and B-GOS groups. The graph indicates that cell cytotoxicity is higher in the B-GOS group compared to the placebo group at all time points.]
IL-6 cytokine was lower following B-GOS compared to placebo at week 10.
Prebiotic impact

- Readdressing the microbial balance
- Reducing signs of inflammation

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<tr>
<th></th>
<th>placebo</th>
<th>B-GOS</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>5wk</td>
</tr>
<tr>
<td><strong>log10 cells/g feces</strong></td>
<td></td>
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</tr>
<tr>
<td><em>Bifidobacterium</em> spp.</td>
<td>9.15 ± 0.35</td>
<td>9.19 ± 0.31</td>
</tr>
<tr>
<td><em>Bacteroides</em> spp.</td>
<td>10.05 ± 0.19</td>
<td>10.12 ± 0.16(^2)</td>
</tr>
<tr>
<td><em>Lactobacillus-Enterococcus</em> spp.</td>
<td>9.11 ± 0.20</td>
<td>9.04 ± 0.24(^6)</td>
</tr>
<tr>
<td><em>Clostridium coccoides-Eubacterium rectale</em></td>
<td>10.26 ± 0.13</td>
<td>10.23 ± 0.12</td>
</tr>
<tr>
<td><em>Clostridium histolyticum</em> group</td>
<td>9.52 ± 0.18</td>
<td>9.54 ± 0.20</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>8.38 ± 0.29</td>
<td>8.48 ± 0.35(^9)</td>
</tr>
<tr>
<td><em>Desulfovibrio</em> spp.</td>
<td>8.15 ± 0.28</td>
<td>8.18 ± 0.30</td>
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Effect on immunity

Immune function markers at 10 weeks

GOS vs Placebo
- IL-6, P<0.001
- IL-10 and TNF-α, P<0.01
- IL-1β, P<0.05

IL-10 (■)
IL-1β (■)
TNF-α (■)
IL-6 (■) – on the right axis
Cells engaged in phagocytosis

\[ \text{% positive} \]

- Placebo
- GOS

Baseline | Week 5 | Week 10
---|---|---

\[ \text{‡ Different from baseline (P<0.001) and placebo (P<0.001)} \]
\[ \text{† Different from baseline (P<0.001) and placebo (P<0.05)} \]
\[ * \text{ Different from baseline (P<0.001), placebo (P<0.001) and 5 weeks (P<0.001)} \]

Baseline – no difference

Effect on immunity

Phagocytic activity against \textit{E. coli}
Effect on NK cell activity

‡ Different from baseline (P<0.001) and 5 weeks (P<0.001)
† Different from baseline (P<0.001) and placebo (P<0.05)
* Different from baseline (P<0.001), 5 weeks (P<0.001) and placebo (P<0.001)
Baseline – no difference

Summarise

- Ageing concerns are increasing
- Prebiotics intervention
- Potentially positive microbial balance
- Increased SCFA
- Reduced signs of immunosenescence and inflammageing
Acknowledgements

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Thank you!

• It’s all about the right balance