Health Benefits of Prebiotics, Probiotics and Synbiotics

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Outline

- Why is a healthy gut so important?
- Do you know your microbiome?
- What are Prebiotics, Probiotics and Synbiotics?
- Clinical Overview of current Science (prebiotics, probiotics and synbiotics)
- Weight Management Results (HOWARU® Shape-\textit{Bifido} 420 + Litesse® polydextrose)
- Conclusions
Gut health
Do you have a healthy gut?

Digestive health not well defined; absence of gastrointestinal disease with normal functioning digestion

Assumed to have a “healthy gut” until a large variety of symptoms occur, often prompting an individual to consult a doctor.

Symptoms often include flatulence, bloating, regurgitation, heartburn, nausea, vomiting, constipation, diarrhea, food intolerance, incontinence, abdominal pain and cramps, loss of appetite, weight loss and blood in stools.

Your Lifestyle and Diet Promote a Healthy Gut- regular exercise, avoidance of chronic stress, as well as use of defined products such as pre- and probiotics as part of a balanced diet, can support gut health.
Measuring functional health of the bowel

Objective measures
- Transit time
- Defecation frequency
- Stool form / Stool dry weight
- Presence of pathogens
- Changes in the microbiota

Subjective measures
- Symptom questionnaires
  - Pain, discomfort, bloating, distension, borborygmi, satisfaction on defecation frequency and stool form
- Effect of bowel function on quality of life

How measured
- Method of data collection
- Frequency
Measuring colonic transit

- Dye; e.g. carmine red
- Radio opaque markers
- 'Smart pill'

Santos et al. 2000
The Microbiome
The Gut Microbiome

Gut microbiota protects against enteropathogens, extracts nutrients and energy from our diets, and contributes to normal immune function.

Our bodies contain 37 trillion human cells - but 100 trillion bacterial cells!

That’s 100,000,000,000,000 microbes!

The healthy gut microbiota is variable and influenced by:
- Diet
- Age
- Genetics
- Antibiotics
- Environment
- Immune System
- Stress

Microbiome in the Big Journals

- Science
- nature
- Cell
- Scientific American
Development of gut microbiota

- Species number accumulates up to ~3 y of life

-> Young and old have a simple microbiota -> less resilient against external stressors

-> external factors effect development (nutrition, probiotics...) -> better resilience

Operational taxonomic unit (OTU)

Microbial stability

Resilience against dysbiosis of the microbiota is likely to be beneficial for health.
The intestine's impact on health. The gastrointestinal tract contributes to health by ensuring digestion and absorption of nutrients, minerals and fluids; by induction of mucosal and systemic tolerance; by defense of the host against infectious and other pathogens; and by signaling from the periphery to the brain.

Bischoff, BMC Medicine 2011 9:24
Probiotics
"Live microbes that, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO 2002; Hill et al., 2014)

Probiotics are

- Safe to consume (identification required)
- Tolerant to acidic conditions in the stomach
- Viable in the host gut
- Beneficial to host health
- Suitable for manufacturing

May benefit health through

- Immune system
- **Intestinal microbiota**

*Lactobacillus* or *Bifidobacterium* most common
Prebiotics
What is a Prebiotic?
-a nondigestible food ingredient that promotes the growth of beneficial microorganisms in the intestines.

Examples include:

Inulin

Xylo-Oligosaccharides (XOS)

Galacto-Oligosaccharides (GOS)

Polydextrose
Current Science

Probiotics
Slow intestinal transit leads to:
- Constipation
- Increased risk for diseases of the colo-rectum
- Reduced quality of life

Triple blind, randomised, placebo controlled
Stratified by:
- Age (25-50/51-65 years)
- Gender

Study product:
- Placebo
  - Microcrystalline cellulose
- Low Dose HN019 $10^9$ CFU/day
- High Dose HN019 Bifido$10^{10}$ CFU/day

Study design cont.

Run-in period

Day-7
Day-6

Day 0

Intervention period

Day 8
Day 14

Daily consumption of capsules with radiopaque markers

Abdominal x-ray, FFQ, DDQ, begin daily supplementation

Daily consumption of capsules with radiopaque markers

Abdominal x-ray, FFQ, DDQ, complete daily supplementation

DDQ = digestive discomfort questionnaire; FFQ = food frequency questionnaire.

Radio opaque markers
### Results:

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Placebo</th>
<th>Low Dose</th>
<th>High Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Upper GI symptoms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>-2%</td>
<td>-12%</td>
<td>-17%</td>
</tr>
<tr>
<td>Regurgitation</td>
<td>-5%</td>
<td>-20%</td>
<td>-24%</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>-12%</td>
<td>-35%</td>
<td>-27%</td>
</tr>
<tr>
<td>Nausea</td>
<td>-7%</td>
<td>-22%</td>
<td>-23%</td>
</tr>
<tr>
<td>Gurgling</td>
<td>-7%</td>
<td>-31%</td>
<td>-16%</td>
</tr>
<tr>
<td><strong>Lower GI symptoms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td>-15%</td>
<td>-32%</td>
<td>-29%</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>-17%</td>
<td>0%</td>
<td>-6%</td>
</tr>
<tr>
<td>Irregular bowel movements</td>
<td>-11%</td>
<td>-25%</td>
<td>-20%</td>
</tr>
<tr>
<td>Flatulence</td>
<td>-8%</td>
<td>-19%</td>
<td>-15%</td>
</tr>
</tbody>
</table>
HOWARU® Bifido (HN019):
- Shortens colonic transit time
- Reduces gastrointestinal symptoms
- Dose dependent effect

Waller et al. 2011
HOWARU® Restore (multi strain blend)  
The clinically proven probiotic for restoring gut health

HOWARU® Restore is a patented probiotic formulation combining top-quality lactobacilli and bifidobacteria strains to address gut stress episodes.

PRODUCT FORMULATION:
A formulation for better benefits:

- *Lactobacillus acidophilus NCFM®*
- *Lactobacillus paracasei Lpc-37™*
- *Bifidobacterium lactis Bi-07™*
- *Bifidobacterium lactis BI-04™*
HOWARU® Restore
Proven efficacy to reduce Antibiotic Associated Diarrhea in adults

AIM OF THE STUDY

This study was designed to determine the dose response effect of HOWARU® Restore formulation on the incidence of AAD and CDAD and severity of gastrointestinal symptoms in adults having antibiotherapy.

PRODUCT FORMULA

L.acidophilus NCFM® ; L.paracasei Lpc-37™ ; B.lactis Bi-07™
B.lactis BI-04™

STUDY DESIGN

Triple blind, randomised, placebo controled

SUBJECTS

Adults in-patients requiring antibiotherapy.

450 PATIENTS DIVIDED IN 3 TREATMENT GROUPS

- Placebo
- HOWARU® Restore low dose (2.5 x 10⁹ CFU/day)
- HOWARU® Restore high dose (10 x 10⁹ CFU/day)
HOWARU® Restore
Significant reduction of Incidence of AAD

Subjects taking the high dose HOWARU® Restore treatment has experienced a 50% reduction in incidence of AAD compared to the placebo group (p<0.005).

Low dose HOWARU® Restore group has shown a 20% reduction compared to placebo group but the results were not statistically significant.

Probiotics reduce symptoms of antibiotic use in a hospital setting: a randomized dose response study; A.Ouwehand et al.; Vaccine: 2013
HOWARU® Restore
Significant reduction of the duration of diarrhea

HOWARU® Restore low dose and high dose have both shown to reduce significantly (p<0.0001) the duration of diarrhea compared to placebo:

- 35% reduction for low dose treatment
- 52% reduction for high dose treatment

In days

Probiotics reduce symptoms of antibiotic use in a hospital setting: a randomized dose response study; A.Ouwehand et al.; Vaccine: 2013
HOWARU® Restore

Significant reduction of the undesirable impacts of antibiotherapy

HOWARU® Restore has shown also some effects in the reductions of undesirable gastrointestinal symptoms during antibiotherapy with:

- A significant reduction of abdominal pain for both groups (p<0.001)
- A significant reduction of bloating for both groups (p=0.03)
- A significant reduction of fever in the high dose group (p=0.02)

Probiotics reduce symptoms of antibiotic use in a hospital setting: a randomized dose response study; A.Ouwehand et al.; Vaccine: 2013
Clostridium difficile is known to cause diarrhea and is particularly troublesome in the elderly, thus having a significant effect in some nursing homes. Therefore, there is a substantial need for a product that can reduce the incidence of this condition.

The high dose of HOWARU® Restore significantly reduced the incidence of CDAD (p=0.02).

Probiotics reduce symptoms of antibiotic use in a hospital setting: a randomized dose response study; A.Ouwehand et al.; Vaccine: 2013
<table>
<thead>
<tr>
<th>Health Condition</th>
<th>Effectiveness of Probiotics</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irritable bowel syndrome (IBS)</td>
<td>Effects seen in overall symptom score, abdominal pain, flatulence, bloating and quality of life</td>
<td>Hoveyda, N., C. Heneghan, et al. 2009\textsuperscript{12}</td>
</tr>
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<td>Hungin, A. P., C. Mulligan, et al. 2013\textsuperscript{11}</td>
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<td>Korterink, J. J., L. Ockeloen, et al. 2014\textsuperscript{13}</td>
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<tr>
<td>Constipation</td>
<td>Reduces intestinal transit time alleviating constipation</td>
<td>Miller, L. E. and A. C. Ouwehand, 2013\textsuperscript{14}</td>
</tr>
<tr>
<td>Infectious diarrhea</td>
<td>Reduction of duration of diarrhea and of defecation frequency.</td>
<td>Applegate, J. A., C. L. Fischer Walker, et al. 2013\textsuperscript{15}</td>
</tr>
<tr>
<td>Antibiotic-associated diarrhea (AAD) and <em>Clostridium difficile</em> infection (CDI)</td>
<td>Reduced risk of AAD and CDI</td>
<td>Pattani, R., V. A. Palda, et al. 2013\textsuperscript{16}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ouwehand, A. C., C. DongLian, et al. 2013\textsuperscript{17}</td>
</tr>
<tr>
<td>Necrotizing enterocolitis (NEC)</td>
<td>Reduction of risk of severe NEC and mortality</td>
<td>AlFaleh, K. and J. Anabrees, 2014\textsuperscript{18}</td>
</tr>
<tr>
<td>Inflammatory bowel diseases (IBD)</td>
<td>Remission prolonged but not induced in ulcerative colitis; Not effective in maintaining remission or preventing relapse in Crohn’s disease</td>
<td>Rahimi, R., S. Nikfar, et al. 2008\textsuperscript{19}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sang, L. X., B. Chang, et al. 2010\textsuperscript{20}</td>
</tr>
</tbody>
</table>
Obesity is a global issue

More than 50% of the world’s 671 million obese live in top 10 obesity countries

1. US
2. China
3. India
4. Russia
5. Brazil
6. Mexico
7. Egypt
8. Germany
9. Pakistan
10. Indonesia

The US, United Kingdom, and Australia are among the high-income countries with large gains in obesity. 2016-2020 Asia is going to take the prize for top growth.

Source: 1) EuroMonitor, 2) Global Industry Analysts Inc, CA, USA; 3) WHO; 4) IHME Institute for Health Metrics and Evaluation
Microbiome and body mass regulation – what is the evidence?

- Microbiota differ in samples from lean and obese individuals

- Less microbial diversity has been identified in obese

- Differences in expression pathways related to body mass regulation between lean and obese microbiome samples

Functional differences in obesity

- Altered satiety signaling and decreased SCFA production; SCFAs have been shown to increase anorectic gut peptide secretion (e.g. GLP-1, PYY)

- Reduced barrier function - Increased bacterial translocation contributing to metabolic endotoxemia and low grade inflammation

Boulangé et al. Genome Medicine, 2016;8:42
Walters et al., FEBS Lett. 2014;588(22): 4223–4233
Ridaura et al. Science. 2013;341(6150)
Turnbaugh et al., Nature. 2009; 457(7228): 480–484
Turnbaugh et al., Nature, 2006;444(7122):1027-31

Probiotics and prebiotics: can they help reverse?

Scientific Documentation

*Bifidobacterium animalis* ssp. *lactis* 420 (B420™) for metabolic health – a decade of research

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<tbody>
<tr>
<td>Cell culture studies: B420™ improves epithelial barrier function</td>
<td>Mouse studies: B420™ improves metabolic health and gut barrier function</td>
<td>Mouse studies: B420™ + Litesse® Ultra improves metabolic health</td>
<td>Clinical study: B420™ with or without Litesse® Ultra controls body fat mass</td>
</tr>
</tbody>
</table>

Amar et al. EMBO Mol Med 2011
Stenman et al. Benef Microbes 2014
Garidou et al. Cell Metab 2015
Stenman et al. EBioMedicine 2016

Scientific Documentation 2008 & 2016: Cell culture studies: B420™ improves epithelial barrier function

Scientific Documentation 2011–2015: Mouse studies: B420™ improves metabolic health and gut barrier function

Scientific Documentation 2015: Mouse studies: B420™ + Litesse® Ultra improves metabolic health

Scientific Documentation 2013–2016: Clinical study: B420™ with or without Litesse® Ultra controls body fat mass
Why B420™?

B420™ supernatant improves epithelial integrity in cell model

Epithelial integrity is a key function of the gastrointestinal tract, and its disturbance is connected to metabolic disorders such as obesity. The finding of improved epithelial integrity by B420™ led to testing it for weight management.

**Why B420™?**

**B420™ ameliorates weight gain in mice**

In mouse studies, B420™ was found to reduce weight gain and the accumulation of fat mass on an unhealthy diet. This provided first proof of concept of B420™ as an aid for weight management.

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Stenman et al. Beneficial Microbes 2014

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* P < 0.05  
** P < 0.01  
** P < 0.0001  
$$$ P = high significance compared to Fat Diet
Polydextrose Scientific evidence – Satiety and weight management

Litesse® (polydextrose) a prebiotic fiber:
- Litesse® is well-tolerated, low calorie, water soluble fiber. Litesse® is better tolerated than almost all low caloric carbohydrates in commercial use (e.g. polyols, inulin).

Clinical studies on Litesse® have shown that:
- Litesse® reduces energy intake at a subsequent meal. The energy intake was not compensated for later in the day (Hull et al 2012; Astbury et al 2013).
- Litesse® increased satiety and decreased after meal feelings of hunger (Hull et al 2012; Olli et al 2015).
- Litesse® increase GLP-1 response after meal (Olli et al 2015). GLP-1 is known to contribute to a decreased food intake.

Systematic review and meta-analysis on Litesse® have shown that:
- The consumption of Litesse® reduces energy intake at a subsequent meal. Furthermore, the reduction in energy intake occurs in a dose-dependent manner (Ibarra et al 2015).
- Litesse® reduces the desire to eat during the satiation period. This may explain partially the reduced energy intake at a subsequent meal (Ibarra et al 2016).
**Fermentability of different prebiotics**

1st generation of prebiotics are rapidly fermented.

Polydextrose is slowly fermented.

<table>
<thead>
<tr>
<th>Proximal colon</th>
<th>Distal colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st generation prebiotics increase fermentation in the proximal colon.</td>
<td>Fermentation of Polydextrose continues throughout the colon, even in the distal colon.</td>
</tr>
</tbody>
</table>

1st generation of prebiotics.

They feed the good bacteria mainly in the first part of the colon.

Polydextrose feeds the good bacteria throughout more of the digestive tract.
Litesse® significantly increases the beneficial intestinal microflora (*Lactobacillus* and *Bifidobacteria*) while significantly reducing the pathogen concentration in the feces (Gram – bacteria).

Litesse® – production of volatile fatty acids

Polydextrose is fermented in the colon producing short chain fatty acids which decreases pH, thus inhibiting pathogen growth.

Increased butyrate promotes the growth of colonic epithelium.

A unique clinical study

Probiotics and prebiotics for controlling body fat mass

- Randomized, double-blind, multicenter study
- Study design and execution follows ICH-GCP
- 225 Finnish participants with BMI 28-34.9, otherwise healthy
- Primary outcome: relative change in body fat mass

Baseline

- Control: microcrystalline cellulose (MCC)
- Probiotic: B420™ + MCC
- Prebiotic: Litesse® Ultra polydextrose
- Synbiotic: Litesse® Ultra + B420™ (HOWARU® Shape)

6 Months

Follow-up: +1 month

Stenman et al. EBioMedicine 2016  ClinicalTrials.gov NCT01978691
The investigational products (once per day)

- **B420™**
  - 12 g microcrystalline cellulose
  - $10^{10}$ CFU/day *B. lactis* 420

- **Litesse® Ultra**
  - 12 g Litesse® Ultra polydextrose

- **Litesse® Ultra + B420™**
  - 12 g Litesse® Ultra polydextrose
  - $10^{10}$ CFU/day *B. lactis* 420

- **Placebo**
  - 12 g microcrystalline cellulose (no energy; less fermented)

Stenman et al. EBioMedicine 2016
The Study Populations, as pre-defined

Randomized (n = 225)

Intention-to-treat (n = 209)
Placebo: 56
Litesse® Ultra: 53
B420™: 48
Litesse® Ultra + B420™: 52

Per protocol (n = 134)
Placebo: 36
Litesse® Ultra: 36
B420™: 25
Litesse® Ultra + B420™: 37

Excluded (n = 16)
Dropped out before the next study visit after baseline

Excluded (n = 75)
- Product non-compliance
- End-of-study visit not completed
- Use of antimicrobials during the intervention

Stenman et al. EBioMedicine 2016
Statistical analyses – two approaches

ANCOVA + Dunnett’s test

Pre-defined Primary analysis

Factorial analysis

Each group is individually compared to placebo.

ANCOVA is the "sum" of all three group-wise tests.

Group-wise tests are corrected for multiple comparisons.

This setting allows analysis by factor, which improves statistical power.
1.4 kg reduction of total Body Fat Mass compared to placebo…

Primary outcome: B420™ + Litesse® Ultra control total body fat mass

Stenman et al. EBioMedicine 2016

Intention to Treat
Overall ANCOVA P=0.46
- Placebo
- Litesse® Ultra
- B420™
- Litesse® Ultra + B420™

Per Protocol
Overall ANCOVA P=0.095
- Placebo
- Litesse® Ultra
- B420™
- Litesse® Ultra + B420™

-4.5% (-1.4 kg) in fat mass
P=0.02

Statistical analyses:
- ANCOVA values
- Factorial analysis
Change in lean body mass and body weight

Improved body composition in Litesse® Ultra+B420™ group

**Lean body mass**
Per Protocol population
Overall ANCOVA P=0.30

- Change in lean body mass, %
  - Placebo: -140 g
  - Litesse® Ultra: +810 g
  - B420™: +810 g

P = 0.012

**Body weight**
Per Protocol population
Overall ANCOVA P=0.13

- Change in body weight, kg
  - Placebo: -1.2 kg
  - Litesse® Ultra: +0.95 kg
  - B420™: -1.2 kg

P = 0.15

**Statistical analyses:**
ANCOVA values
Factorial analysis

Stenman et al. EBioMedicine 2016
B420™ alone or in combo with Litesse® Ultra reduces energy intake

Stenman et al. EBioMedicine 2016
**Effect concentrated to abdominal area**

Stenman et al. EBioMedicine 2016
Summary of results-new approach to weight management

**Litesse Ultra + B420:**
- Control of **body fat mass**, especially in abdominal region
- Normalized **energy balance** (-250 kcal/d)
- Reduced **waist circumference**
- Increased **lean body mass**

**B420:**
- No significant difference in body fat mass in primary analysis
- Post hoc factorial analysis:
  - Maintained **body fat mass** and **body weight**
  - Reduced **waist circumference**
- Normalization of **energy balance** (-300 kcal/day)

Stenman et al. EBioMedicine 2016

* As compared to placebo
Clinical study team

Principal Investigator
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Gut microbiota sequencing:
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Ashley Hibberd
Wesley Morovic
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Conclusions

In order to maintain a healthy digestive system, there are lifestyle habits like exercise, eating a balanced diet and avoiding too much stress which are important.

Consumption of pre- and probiotics can help contribute to a healthy gut by promoting a balanced microbiome.

The combination of pre- and probiotics may promote a better metabolic state and help with weight management.
DuPont Nutrition & Health combines in-depth knowledge of food and nutrition with current research and expert science to deliver unmatched value to the food, beverage and dietary supplement industries.

We are innovative solvers, drawing on deep consumer insights and a broad product portfolio to help our customers turn challenges into high-value business opportunities