Harnessing the gut microbiome and probiotics for better health in a broader population

Anders Henriksson, Ph.D
Principal Application Scientist
DuPont Nutrition & Health
Impact of the microbiota

- Cause cavities and gingivitis
- Resolve or cause chronic ear infection
- Influence liver health
- Influence small intestinal health
- Toxify or detoxify
- Train & modulate immune system
- Production of antimicrobials and inhibitions pathogens
- Modulate the gut-brain connection
- Modulate the HPA axis
- Reduce hypersensitivity
- Reduce asthma
- Cause or revolve GERD
- Influence colonic health
- Influence vaginal health
- Prostate health
- Underlie arthritis & autoimmune

GERD: gastroesophageal reflux disease

Increase or reduce risk of obesity & diabetes
Microbiota of infants and toddlers provide a window of opportunity

Fetal and postnatal time up to 2-3 years of age are crucial for development of microbiota and immunity
- Microbiota reaches adult type at 3 years of age and immunity at 12 years of age

Source: Graph based on the publications: Yatsunenko et al., 2012 Nature; Kollmann et al. 2012 Immunity
Early intervention with probiotics: Effect on the prevalence of eczema at 2, 4, and 6 years

AIM OF THE STUDY

To understand if the daily intake of *Lactobacillus rhamnosus* HN001 would reduce the incidence and the severity of eczema in children.

STUDY DESIGN

Pregnant mothers treated daily from ~5 weeks pre-term to 6 months post-term for breastfeeding mothers. Infants treated daily from birth to 24 months old — treatments given as supplement to infant feeds (breast milk, infant formula, weaning food). Health assessment was done at 2, 4, and 6 years.

SUBJECTS

Infants with family history of allergy

Approx 150 infants/children/treatment group

- Placebo
- *L. rhamnosus* HN001 at dose of 6 billion per day ($6 \times 10^9$ cfu/day)
Long term study

A Prenatal & Postnatal Treatment

- The following outcomes were assessed at 2, 4, and 6 years of age
  - **Eczema prevalence** was evaluated by
    - using SCORing Atopic Dermatitis (SCORAD) cut off =>10 (to differentiate from rash)
  - **Atopy** was assessed by using Australasian Society of Clinical Immunology and Allergy guidelines.
    - Skin prick tests were done against egg white, peanut, cow’s milk, cat pelt, D.pteronyssinus and mixed grass pollen
  - **Wheeze and rhinoconjunctivitis** was assessed by using International Study of Asthma and Allergies in Childhood criteria.
    - Questionnaire

31% lower cumulative prevalence of eczema over 6 years
[Atopic eczema severity diagnosis tool (SCORAD)]

- Significant reduction at 2 years ➔ 43% (p=0.009)
- The effect continues over 6 years ➔ 31% lower cumulative prevalence (p= 0.04)

Study showed that the *Lactobacillus rhamnosus* HN001 group experienced a 31% reduction in the cumulative prevalence of SCORAD ≥10 points over 6% compared to placebo (Hazard Ratio 0.69; p=0.04).

31% lower prevalence of allergic sensitization over 6 years

By using skin prick tests children in HN001 group had a significantly lower cumulative prevalence of allergic sensitization compared with children taking placebo (HR 0.69; p=0.04)

Skin prick tests measure an immune system reaction towards the allergen.
- Allergens bind to IgE antibodies on immune cells beneath the skin that release inflammatory mediators and cause the typical redness and swelling of the skin.

62% reduced risk of rhinitis and red eyes over 4 years

- Clinical diagnosis (The International Study of Asthma and Allergies in Childhood questionnaire) was used to assess the prevalence of current rhinoconjunctivitis. At 4 years of age, the children in HN001 group had a 62% lower relative risk of having rhinoconjunctivitis (rhinitis and red eyes) (p=0.02).

Impact of the microbiota

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- Prostate health
- Underlie arthritis & autoimmune

GERD: gastroesophageal reflux disease

Increase or reduce risk of obesity & diabetes
Probiotics and antibiotic associated diarrhea

Figure 3

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Probiotics</th>
<th>Control</th>
<th>Risk Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Weight</td>
</tr>
<tr>
<td>2.7.1 L. rhamnosus GG</td>
<td>3</td>
<td>61</td>
<td>58</td>
</tr>
<tr>
<td>Anvola et al., 1999</td>
<td>7</td>
<td>93</td>
<td>95</td>
</tr>
<tr>
<td>Vanderhoef et al., 1999</td>
<td>154</td>
<td>153</td>
<td>95</td>
</tr>
<tr>
<td>Total events</td>
<td>10</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: Tau^2 = 0.00, Ch^2 = 0.02, df = 1 (P = 0.89); P = 0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 3.59 (P = 0.0003)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.7.2 S. bouardi</td>
<td>7</td>
<td>127</td>
<td>12</td>
</tr>
<tr>
<td>Erdede et al., 2004</td>
<td>14</td>
<td>204</td>
<td>28</td>
</tr>
<tr>
<td>Duman et al., 2005</td>
<td>9</td>
<td>62</td>
<td>19</td>
</tr>
<tr>
<td>Cindoruk et al., 2007</td>
<td>11</td>
<td>80</td>
<td>24</td>
</tr>
<tr>
<td>Total events</td>
<td>48</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: Tau^2 = 0.00, Ch^2 = 2.62, df = 4 (P = 0.64); P = 0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 5.45 (P &lt; 0.00001)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.7.3 L. acidophilus La-5 + B. lactis Bi-12</td>
<td>19</td>
<td>198</td>
<td>26</td>
</tr>
<tr>
<td>De Vrese et al., 2011</td>
<td>228</td>
<td>227</td>
<td>227</td>
</tr>
<tr>
<td>Total events</td>
<td>23</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Heterogeneity: Tau^2 = 0.00, Ch^2 = 0.54, df = 1 (P = 0.46); P = 0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: Z = 0.89 (P = 0.37)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total events</td>
<td>972</td>
<td>929</td>
<td>100.0%</td>
</tr>
<tr>
<td>Test for subgroup differences: Ch^2 = 6.30, df = 2 (P = 0.04), P = 0.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Efficacy results of probiotic use: eight RCTs by three probiotic subgroups (outcome: incidence of antibiotic-associated diarrhea (AAD)).

Blaabjerg et al (2017) Antibiotics. 6:21
Probiotics in management of on Antibiotic Associated Diarrhea (AAD) 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.

STUDY DESIGN
Triple blind, randomised, placebo controled

SUBJECTS
Adults in-patients requiring antibiotherapy.

450 PATIENTS DIVIDED IN 3 TREATMENT GROUPS COMPLETED THE STUDY
• Placebo
• Combination of 4 probiotic strains at $4.17 \times 10^9$ CFU/day (low dose)
• Combination of the same 4 strains at $1.70 \times 10^{10}$ CFU/day (high dose)
Probiotics in management of on Antibiotic Associated Diarrhea (AAD) in adults - incidence

- Subjects taking the high dose experienced a 50% reduction in incidence of AAD compared to the placebo group (p<0.005).
- Low dose group has shown a 20% reduction compared to placebo group but the results were not statistically significant.

Ouwehand et al (2014) Vaccine vol 32
Influence small intestinal health

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Increase or reduce risk of obesity & diabetes

GERD: gastroesophageal reflux disease
DIABETES

DIABETES IS ON THE RISE

422 MILLION ADULTS HAVE DIABETES

That’s 1 person in 11

3.7 million deaths due to diabetes and high blood glucose

1.5 million deaths caused by diabetes

www.who.int/diabetes/global-report
Many types of Diabetes

**TYPE 1 DIABETES**
Body does not produce enough insulin

**TYPE 2 DIABETES**
Body produces insulin but can’t use it well

**GESTATIONAL DIABETES**
A temporary condition in pregnancy

www.who.int/diabetes/global-report
Gut-derived metabolic endotoxemia – the hypothesis

Can this be reversed?

High fat diet

Increased intestinal permeability

LPS entering the circulation

Tissue inflammation

Insulin resistance

Metabolic syndrome, Type 2 diabetes
Akkermansia muciniphila and metabolic health

Inflammation and onset of type 2-diabetes, Metabolic syndrome
High levels of *Akkermansia muciniphila* supports good metabolic health

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**Table 1** Comparison between clinical variables categorised into *Akkermansia muciniphila* abundance groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Akk LO (N=24)</th>
<th>Akk HI (N=25)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>19 (79.2)</td>
<td>22 (88.0)</td>
<td>0.4</td>
</tr>
<tr>
<td>M</td>
<td>5 (20.8)</td>
<td>3 (12.0)</td>
<td></td>
</tr>
<tr>
<td>Glucose homoeostasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.4 (0.1)</td>
<td>5.2 (0.1)</td>
<td>0.02</td>
</tr>
<tr>
<td>Insulin (μIU/mL)</td>
<td>11.3 (0.9)</td>
<td>8.9 (0.9)</td>
<td>0.03</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.5 (0.1)</td>
<td>1.2 (0.1)</td>
<td>0.03</td>
</tr>
<tr>
<td>Disease index</td>
<td>−9.2 (1.0)</td>
<td>−6.0 (1.1)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Management of diabetes

Future opportunities with Akkermansia muciniphila

Opportunities with currently available probiotics
Early pregnancy probiotic supplementation with *Lactobacillus rhamnosus* HN001 may reduce the prevalence of gestational diabetes mellitus: a randomised controlled trial

Kristin L. Wickens1, Christine A. Barthow2, Rinki Murphy2, Peter R. Abels1,3, Robyn M. Maude4, Peter R. Stone2, Edwin A. Mitchell2, Thorsten V. Stanley1,3, Gordon I. Purdie1, Janice M. Kang1, Fiona E. Hood1, Judy L. Rowden1, Phillipa K. Barnes1, Penny F. Fitzharris5 and Julian Crane1

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3Capital and Coast DHB, Wellington 6021, New Zealand
4Victoria University, Wellington 6021, New Zealand
5Auckland Hospital, Auckland 1142, New Zealand

(Submitted 22 August 2016 – Final revision received 10 January 2017 – Accepted 31 January 2017 – First published online 2 April 2017)

Abstract

The study aims to assess whether supplementation with the probiotic *Lactobacillus rhamnosus* HN001 (HN001) can reduce the prevalence of gestational diabetes mellitus (GDM). A double-blind, randomised, placebo-controlled parallel trial was conducted in New Zealand (NZ) (Wellington and Auckland). Pregnant women with a personal or partner history of gestational diabetes were randomised at 14–16 weeks' gestation to receive HN001 (6 × 10^9 colony forming units (CFU) in 212 mL) or placebo (212 mL) daily. GDM at 24–30 weeks was assessed using the definition of the International Association of Diabetes and Pregnancy Study Groups (IADPSG) (fasting plasma glucose ≥5.1 mmol/L, or 1h post 75 g glucose level at 3.0 mmol/L or at 2h 2.85 mmol/L and NZ definition (fasting plasma glucose ≥5.5 mmol/L or 2h post 7.5 g glucose at ≥5 mmol/L). All analyses were intention-to-treat. A total of 184 (87%) women took HN001 and 189 (90%) women took placebo. There was a trend towards lower relative rates (RR) of GDM (IADPSG definition) in the HN001 group, 0.59 (95% CI 0.32, 1.00) (*P* = 0.08). HN001 was associated with lower rates of GDM in women aged 23–35 years (RR 0.51; 95% CI 0.31, 0.81, *P* = 0.009) and women with a history of GDM (RR 0.00; 95% CI 0.00, 0.66, *P* = 0.004). These rates did not differ significantly from those of women without these characteristics. Using the NZ definition, GDM prevalence was significantly lower in the HN001 group, 2.1% (95% CI 0.6%, 5.2%), vs. 6.5% (95% CI 3.5%, 10.9%) in the placebo group (*P* = 0.05). HN001 supplementation from 14 to 16 weeks' gestation may reduce GDM prevalence, particularly among older women and those with previous GDM.

Key words: Randomised controlled trials; Probiotics: *Lactobacillus rhamnosus* HN001; Gestational diabetes mellitus

Lifestyle factors such as changes in patterns of food consumption with economic development have led to the well-recognised and increasing problems of obesity and associated diseases, including gestational diabetes mellitus (GDM). Both in New Zealand (NZ) and...
**Lactobacillus rhamnosus HN001™ and prevalence of GDM: Trial design in brief**

- **Placebo group**
  - n = 211
  - Capsules with maltodextrin from 12-16 weeks gestation until 6 months post birth in case of still breast feeding

- **Probiotic group**
  - n = 212
  - Capsules with 6.0E+09 CFU of HN001™ from 12-16 weeks gestation until 6 months post birth in case of still breast feeding

Assessment of gestational diabetes at 24-30 weeks gestation.
Prevalence of Gestational Diabetes

<table>
<thead>
<tr>
<th></th>
<th>HN001</th>
<th></th>
<th>Placebo</th>
<th></th>
<th>RR</th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prevalence (%)</td>
<td>95% CI (%)</td>
<td>Prevalence (%)</td>
<td>95% CI (%)</td>
<td>RR</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>IADPSG* (n 373)</td>
<td>8.2 (15/184)</td>
<td>4.6, 13.1</td>
<td>13.8 (26/189)</td>
<td>9.2, 19.5</td>
<td>0.59</td>
<td>0.32, 1.08</td>
<td>0.08</td>
</tr>
<tr>
<td>NZ† (n 394)</td>
<td>2.1 (4/194)</td>
<td>0.6, 6.5</td>
<td>6.5 (13/200)</td>
<td>3.5, 10.9</td>
<td>0.32</td>
<td>0.11, 0.96</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Effect on fasting glucose levels

<table>
<thead>
<tr>
<th></th>
<th>HN001</th>
<th></th>
<th>Placebo</th>
<th></th>
<th></th>
<th>Difference in mean</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>95% CI</td>
<td>Mean</td>
<td>95% CI</td>
<td>Difference in mean</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>Fastng (mmol/l)</td>
<td>4.32</td>
<td>4.27, 4.37</td>
<td>4.40</td>
<td>4.34, 4.46</td>
<td>-0.08</td>
<td>-0.15, 0.00</td>
<td>0.048</td>
</tr>
</tbody>
</table>

HN001, Lactobacillus rhamnosus HN001.
* Fasting ≥5.1 mmol/l, 1 h ≥10 mmol/l, 2 h ≥8.5 mmol/l.
† Fasting ≥5.5 mmol/l, 2 h ≥9 mmol/l.
Results suggest that benefits are greatest in mothers > 35 years of age and in mothers with a previous history of GDM

<table>
<thead>
<tr>
<th>Influence of age and previous history of GDM</th>
<th>Prevalence of GDM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HN001™</td>
<td>Placebo</td>
</tr>
<tr>
<td>Influence of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 35 years</td>
<td>7.1%</td>
<td>22.9%</td>
</tr>
<tr>
<td>&lt; 35 years</td>
<td>8.8%</td>
<td>8.5%</td>
</tr>
<tr>
<td>Previous History</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With previous history</td>
<td>0%</td>
<td>87%</td>
</tr>
<tr>
<td>No previous history</td>
<td>6.2%</td>
<td>12.3%</td>
</tr>
</tbody>
</table>

Wickens at al. 2017 British Journal of Nutrition
Research Paper

Probiotic With or Without Fiber Controls Body Fat Mass, Associated With Serum Zonulin, in Overweight and Obese Adults—Randomized Controlled Trial

Lotta K. Stenman a,⁎, Markus J. Lehtinen a, Nils Meland b, Jeffrey E. Christensen c, Nicolas Yeung d, Markku T. Saarinen e, Michael Courtney f, Rémy Burcelin g, Marja-Leena Lähdeoaho d, Jüri Linros d, Dan Apter h, Mika Schemir i, Hilde Kloster Smerud a, Aila Rissanen a, Sampo Lahtinen a

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b Department of Clinical Biochemistry, University of Helsinki, Helsinki, Finland
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i Department of Clinical Biochemistry, University of Helsinki, Helsinki, Finland

Abstract

Background: The gut microbiota is interlinked with obesity, but direct evidence of effects of its modulation on body fat mass is still scarce. We investigated the possible effects of Bifidobacterium animalis subsp. lactis 420 (B420) and the dietary fiber Litesse® Ultra polydextrose (LU) on body fat mass and other obesity-related parameters.

Methods: 225 healthy volunteers (healthy, BMI 28–34.9) were randomized into four groups (1:1:1:1; using a computer-generated sequence, for 6 months of double-blind, parallel treatment: 1) Placebo, microcrystalline cellulose, 12 g/d; 2) LU, 12 g/d; 3) B420, 10^10 CFU/d in microcrystalline cellulose, 12 g/d; 4) LU + B420, 12 g + 10^10 CFU/d. Body composition was monitored with dual-energy X-ray absorptiometry, and the primary outcome was relative change in body fat mass, comparing treatment groups to Placebo. Other outcomes included anthropometric measurements, food intake and blood and fecal biomarkers. The study was registered in ClinicalTrials.gov (NCT01978951).

Findings: There were marked differences in the results of the Intention-To-Treat (ITT; n = 209) and Per Protocol (PP; n = 134) study populations. The PP analysis included only those participants who completed the intervention.
**Akkermansia** is more abundant in human subjects consuming B-420™

- B-420™ impacted many species of the human gut microbiota; including, significantly higher relative abundance of **Akkermansia muciniphila** in B-420™ subjects.

- Abundance of **Akkermansia muciniphila**, a mucin-degrading bacterium that resides in the mucus layer, is generally associated with improved metabolic health including body weight, glucose tolerance and intestinal permeability.

- **A. muciniphila** has been identified as inversely correlated with body weight gain in pregnant women.

- **A. muciniphila** controlled gut barrier function, fat mass storage, and glucose tolerance in obese and type 2 diabetic mice.

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1 Hibberd et al. 2018. Beneficial Microbes (under peer review)
2 SantaCruz et al. 2010. British Journal of Nutrition
3 Everard et al., 2013. Proceedings of the National Academy of Sciences of the United States of America
4 Dao et al., 2016. Gut
Harnessing the gut microbiome and probiotics, for better health in a broader population
Probiotics in

Treatment

Prevention

“The GOOD physician treats the DISEASE; the GREAT physician treats the PATIENT who has the disease.”

Sir William Osler
Early intervention with probiotics: Effect on the prevalence of eczema at 2, 4, and 6 years

**AIM OF THE STUDY**
To understand if the daily intake of *Lactobacillus rhamnosus* HN001 would reduce the incidence and the severity of eczema.

**STUDY DESIGN**
Pregnant mothers treated daily from 15 weeks post-term for breastfeeding mothers, infants treated daily from birth to 24 months old — treatments given as supplement to infant formula. Health assessment was done at 2, 4, and 6 years.

**SUBJECTS**
Infants with family history of allergy
- Group 1: Infants children/treatment group
  - Placebo
  - *L. rhamnosus* HN001 at dose of 1 billion per day (25 mg)

Probiotics in management of antibiotic associated diarrhea (AAD) in adults

**AIM OF THE STUDY**
This study was designed to determine the dose response effect of HOWAR® Restore formulation on the incidence of AAD and COAD and severity of gastrointestinal symptoms in adults having antibiotic therapy.

**STUDY DESIGN**
Triple blind; randomized, placebo controlled

**SUBJECTS**
Adults inpatients requiring antibiotic therapy
- 250 patients divided into 3 treatment groups completed the study
  - Placebo
    - Combination of 4 probiotic strains at 4.17 x 10^9 CFU/day (low dose)
    - Combination of the same 4 strains at 1.70 x 10^10 CFU/day (high dose)

*Lactobacillus rhamnosus* HN001™ and prevalence of GDM: Trial design in brief

- **Placebo group**
  - n = 211
  - Capsules with medication from 12-16 weeks gestation until 6 months postbirth in case of still breast feeding

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  - Capsules with 5.95 x 8.10 (12) of HN001™ from 12-16 weeks gestation until 6 months postbirth in case of still breast feeding

**Assessment of gestational diabetes at 24-30 weeks gestation**
Probiotics in

**Treatment**

“The GOOD physician treats the DISEASE; the GREAT physician treats the PATIENT who has the disease.”

Sir William Osler

**Prevention**

Compounding
Opportunities with different delivery formats
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.

Thank You