DuPont™ Danisco®

December 6, 2018

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

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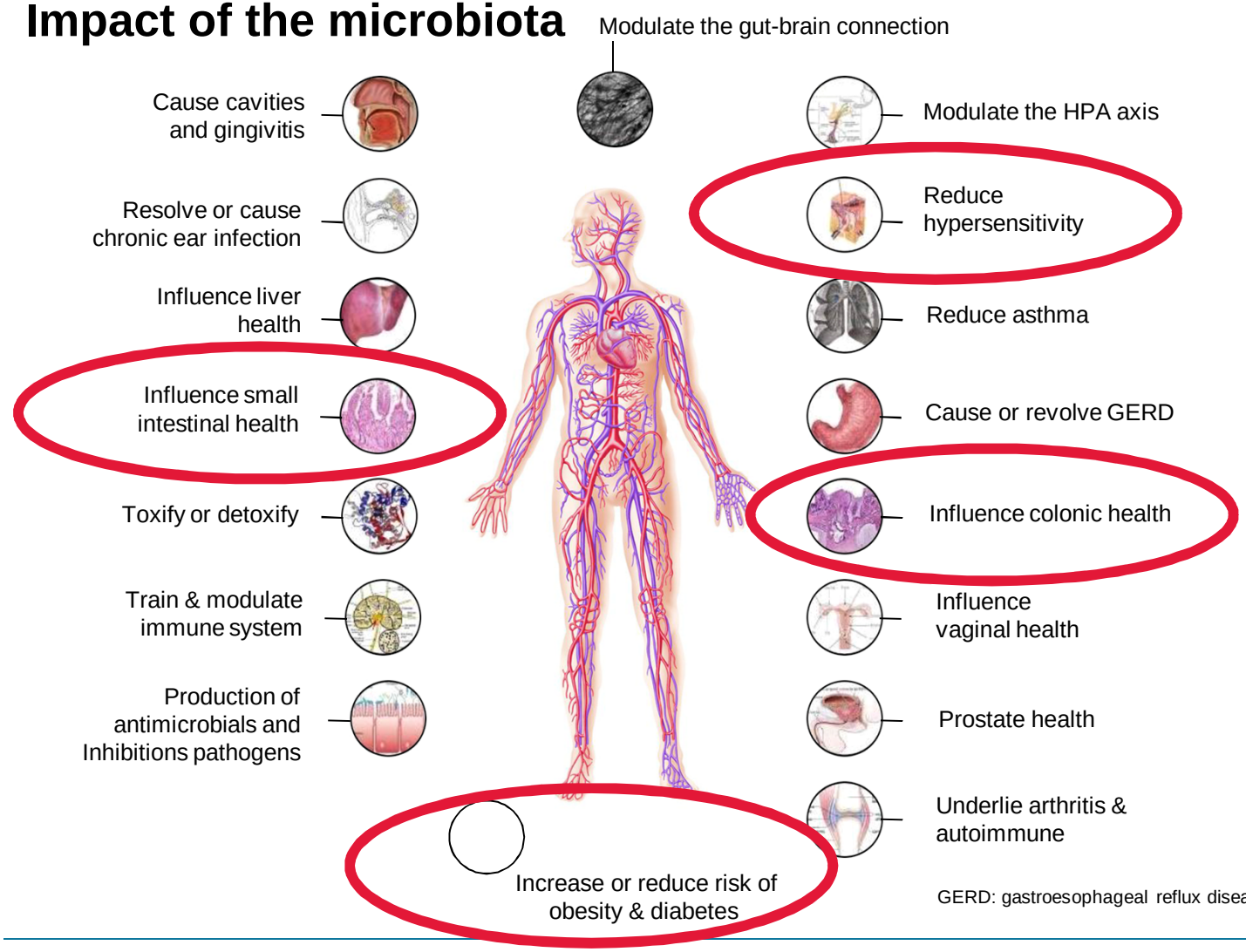
Principal Application Scientist

DuPont Nutrition & Health

DANISCO.

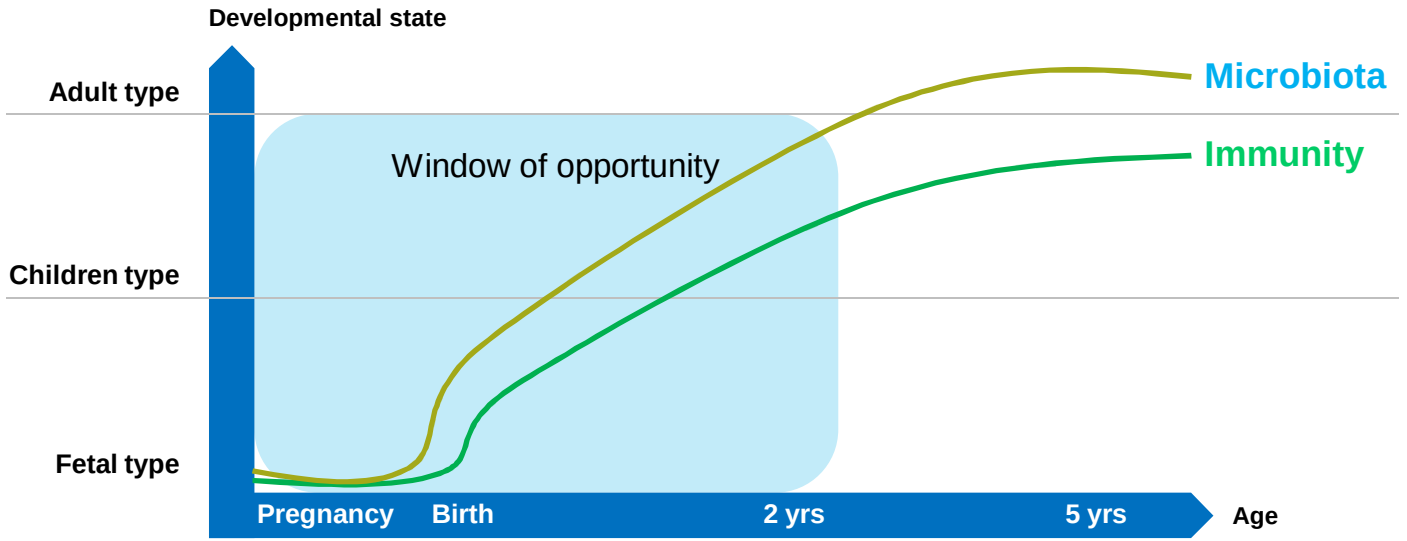
Impact of the microbiota



GERD: gastroesophageal reflux disease

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


Atopic dermatitis and skin disease

A differential effect of 2 probiotics in the prevention of eczema and atopy: A double-blind, randomized, placebo-controlled trial

ORIGINAL ARTICLE Epidemiology of Allergic Disease

A protective effect of *Lactobacillus rhamnosus* HN001 against eczema in the first 2 years of life persists to age 4 years

Clinical & Experimental Allergy

ORIGINAL ARTICLE Epidemiology of Allergic Disease

Early supplementation with *Lactobacillus rhamnosus* HN001 reduces eczema prevalence to 6 years: does it also reduce atopic sensitization?

Clinical & Experimental Allergy

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 (**$6*10^9$** 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

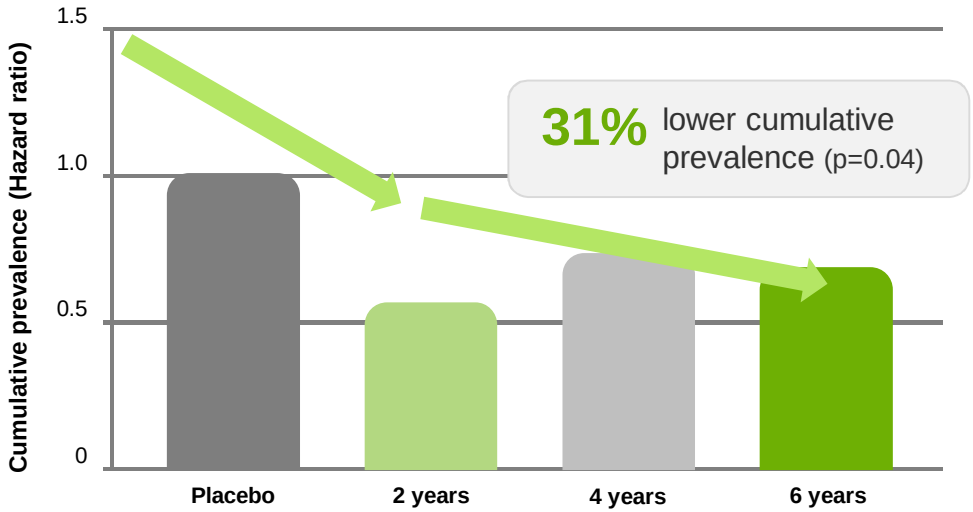
Source: Wickens et al. 2008; Wickens et al. 2012; Wickens et al. 2013;

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)

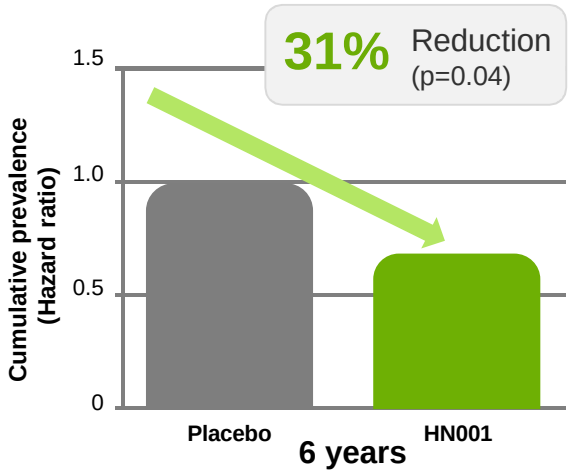
Study showed that the ***Lactobacillus rhamnosus*** **HN001** group experienced a 31% reduction in the ***cumulative prevalence*** of SCORAD ≥10 points over 6% compared to placebo (Hazard Ratio 0.69; p=0.04).



Source: Wickens et al. 2008; Wickens et al. 2012; Wickens et al. 2013;

31% lower prevalence of allergic sensitization over 6 years

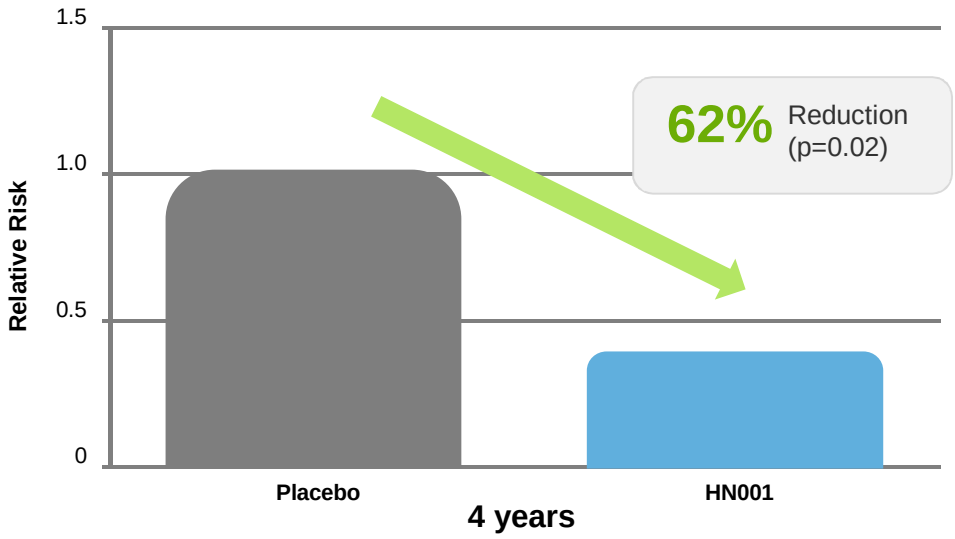
- By using skin prick tests children in HN001 group had a significantly lower cumulative prevalence of allergic sensitization compared with children taking placebo (HR 0.69; p=0.04)
- Skin prick tests measure an immune system reaction towards the allergen.
  - Allergens bind to IgE antibodies on immune cells beneath the skin that release inflammatory mediators and cause the typical redness and swelling of the skin.



Source: Wickens et al. 2013;

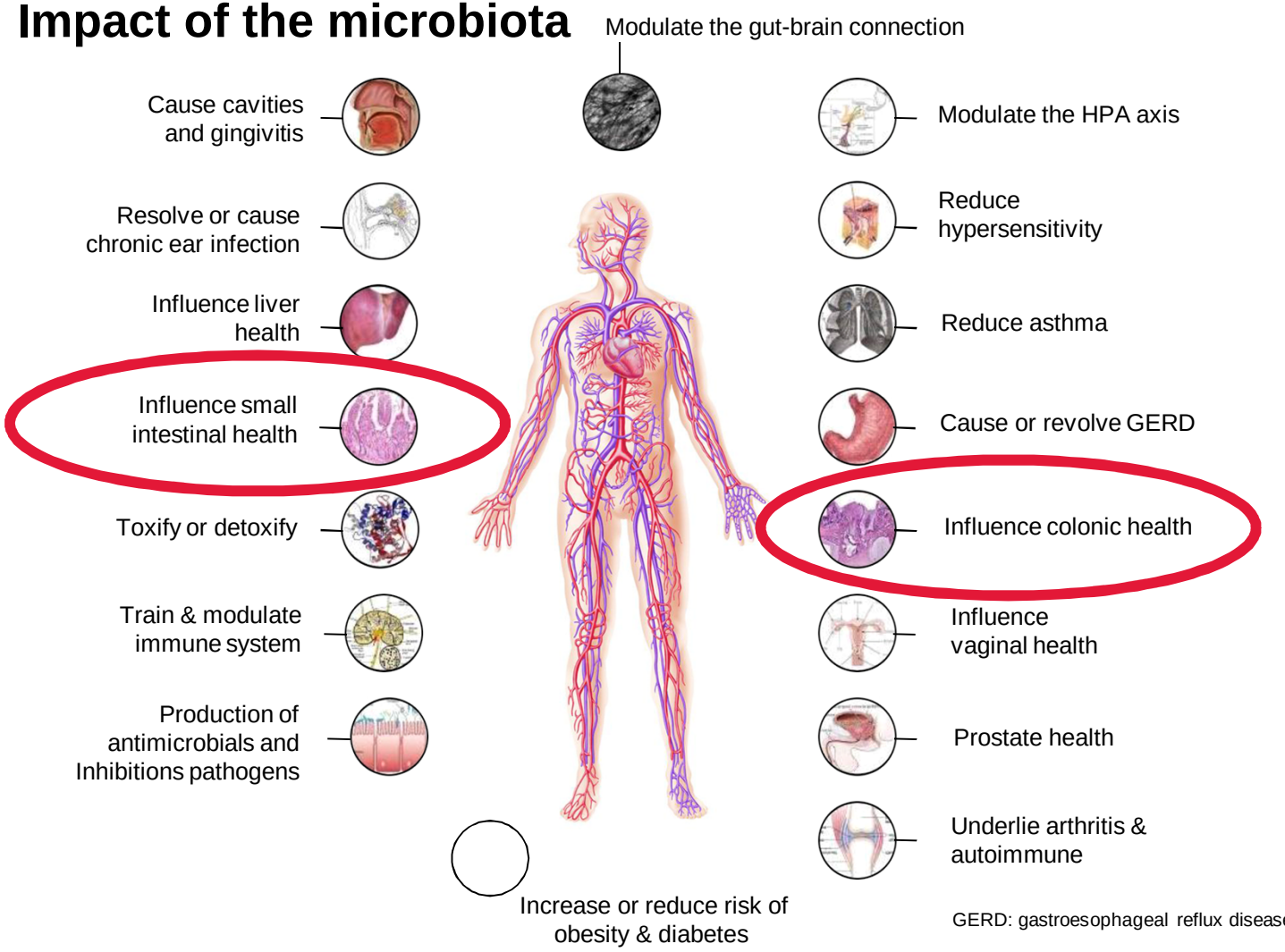
62% reduced risk of rhinitis and red eyes over 4 years

- 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).



Source: Wickens et al. 2012

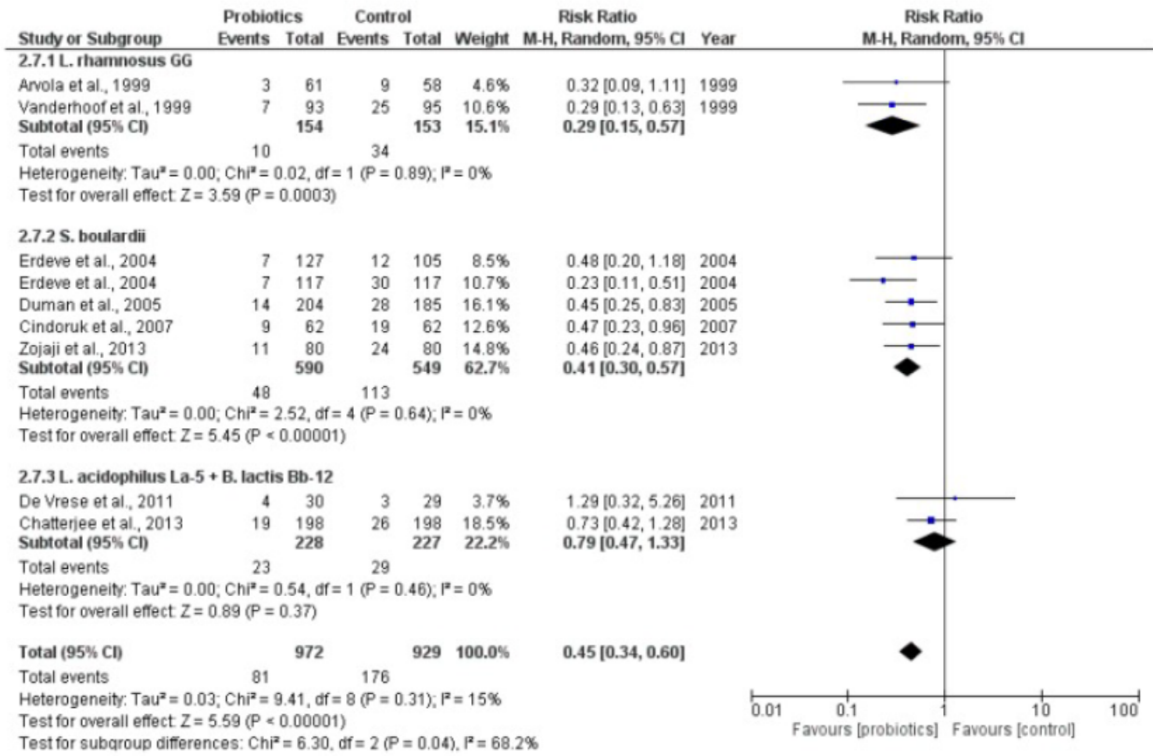
Impact of the microbiota



GERD: gastroesophageal reflux disease

Probiotics and antibiotic associated diarrhea

Figure 3



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

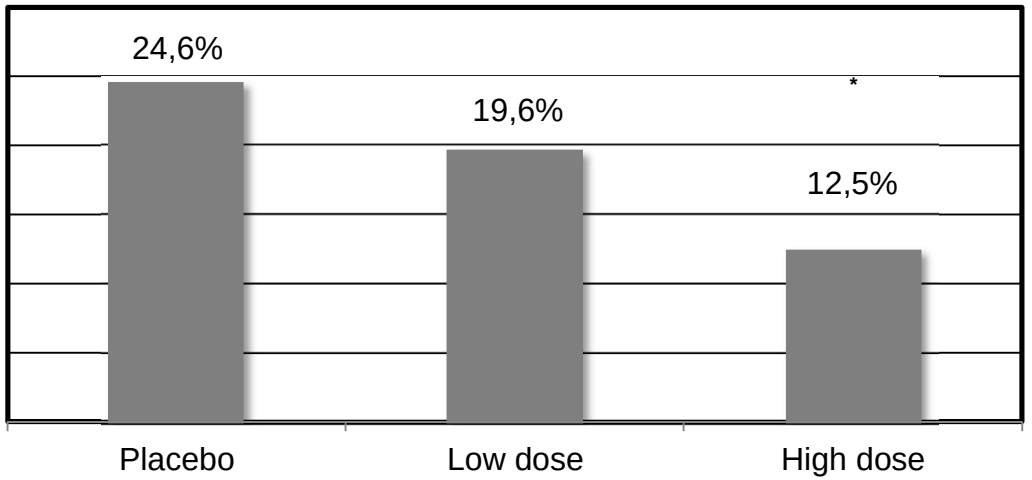
- Placebo
- Combination of 4 probiotic strains at 4.17 x 10^9 CFU/day (low dose)
- Combination of the same 4 strains at 1.70 x 10^{10} CFU/day (high dose)

Vaccine

Probiotics reduce symptoms of antibiotic use in a hospital setting: A randomized dose response study

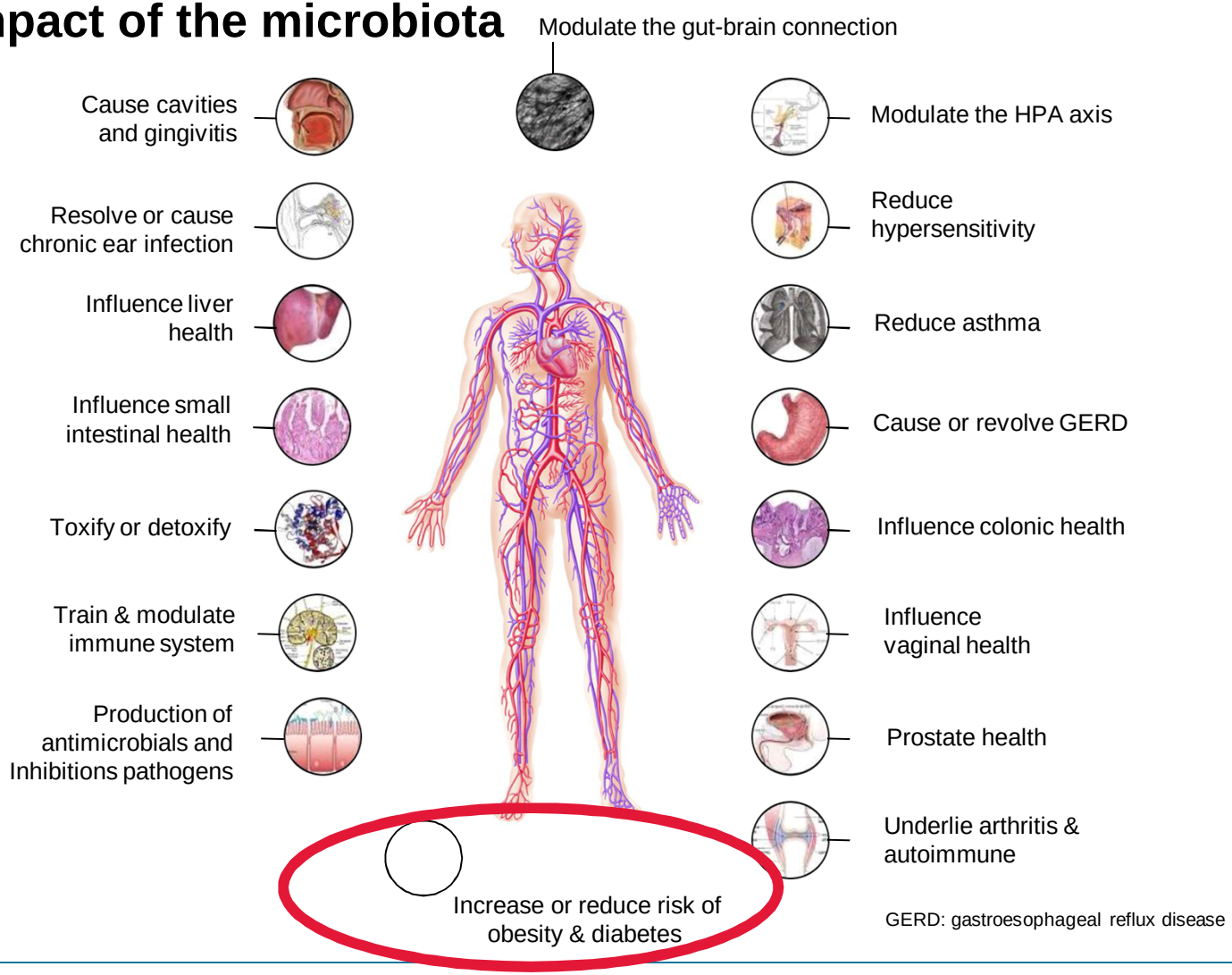
Probiotics in management of on Antibiotic Associated Diarrhea (AAD) in adults - incidence

- 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

Impact of the microbiota



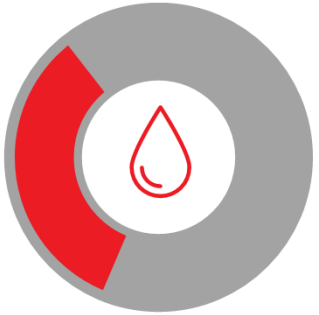
GERD: gastroesophageal reflux disease

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

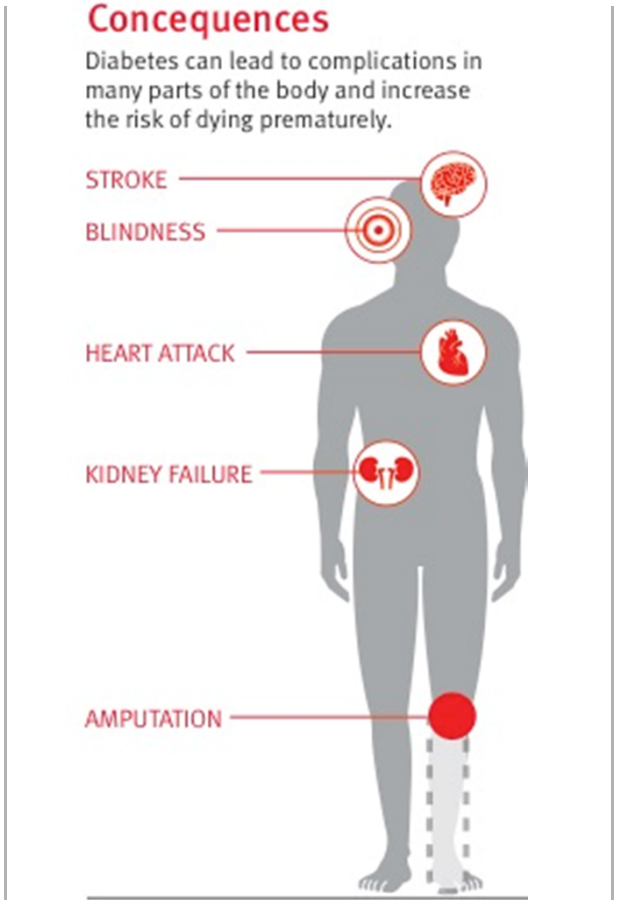
www.who.int/diabetes/global-report

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

High fat diet

Increased intestinal permeability

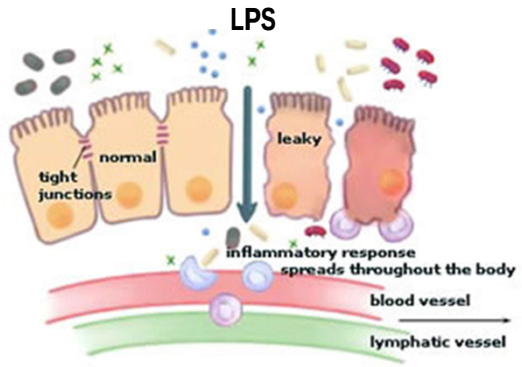
LPS entering the circulation

Tissue inflammation

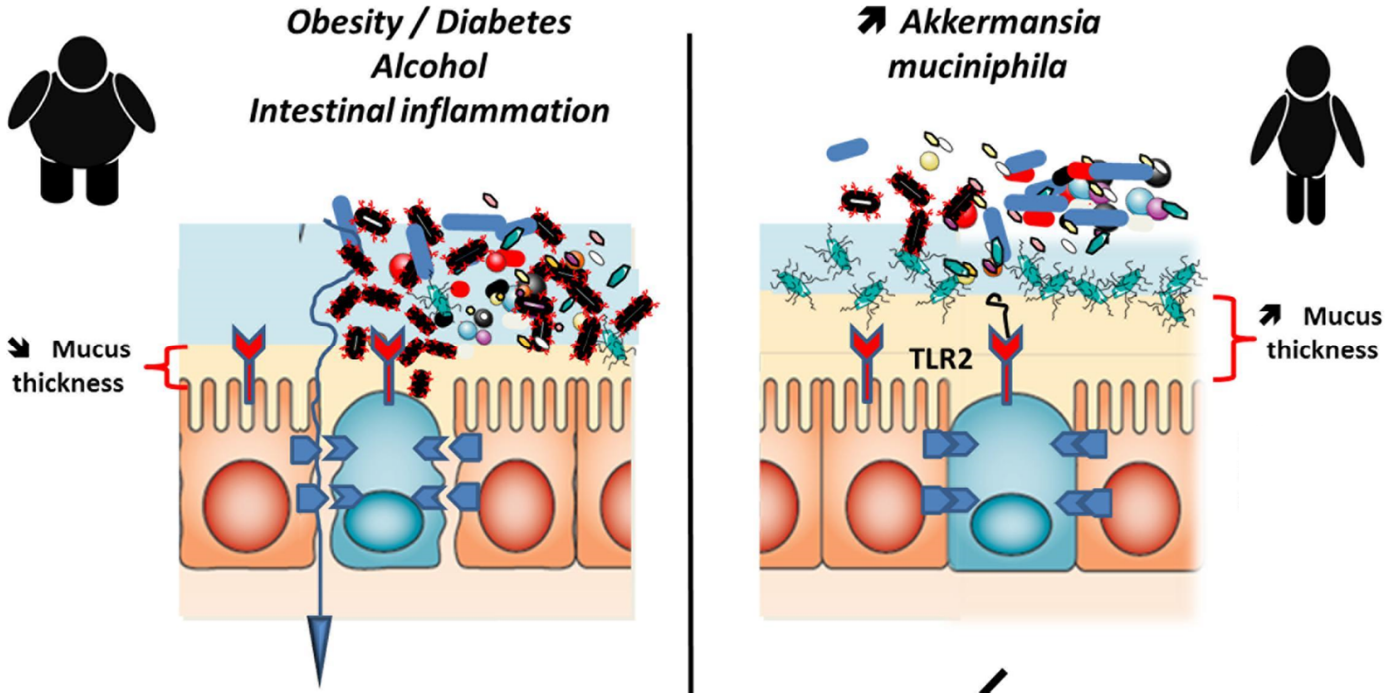
Insulin resistance

Metabolic syndrome, Type 2 diabetes

Can this be reversed?



Akkermansia muciniphila and metabolic health



Inflammation and onset of type 2-diabetes, Metabolic syndrome

High levels of *Akkermansia muciniphila* supports good metabolic health

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

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

Adapted from Dao et al (2016) Gut 65:426-436

Management of diabetes

British Journal of Nutrition (2017), 117, 804–813 doi:10.1017/S0007114517000289

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

Kristin L. Wickens[1]*, Christine A. Barthow[1], Rinki Murphy[2], Peter R. Abels[1,3], Robyn M. Maude[4], Peter R. Stone[2], Edwin A. Mitchell[2], Thorsten V. Stanley[1,3], Gordon L. Purdie[1], Janice M. Kang[1], Fiona E. Hood[1], Judy L. Rowden[2], Phillipa K. Barnes[1], Penny F. Fitzharris[5] and Julian Crane[1]

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Abstract

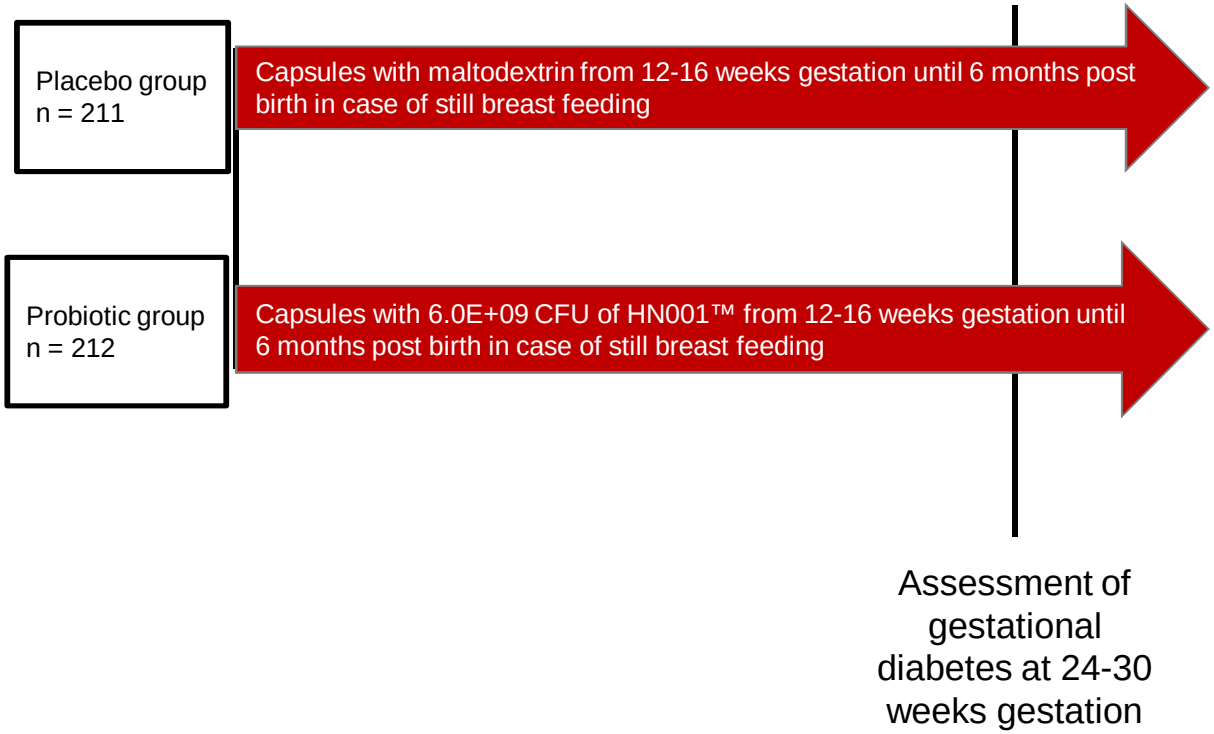
The study aims to assess whether supplementation with the probiotic *Lactobacillus rhamnosus* HN001 (HN001) can reduce the prevalence of gestational diabetes mellitus (GDM). A double-blind, randomised, placebo-controlled parallel trial was conducted in New Zealand (NZ) (Wellington and Auckland). Pregnant women with a personal or partner history of atopic disease were randomised at 14–16 weeks' gestation to receive HN001 (6×10^9 colony-forming units) (*n* 212) or placebo (*n* 211) 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 1 h post 75 g glucose level at ≥10 mmol/l or at 2 h ≥8·5 mmol/l) and NZ definition (fasting plasma glucose ≥5·5 mmol/l or 2 h post 75 g glucose at ≥9 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, 1·08) ($P = 0·08$). HN001 was associated with lower rates of GDM in women aged ≥35 years (RR 0·31; 95 % CI 0·12, 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), *v.* 6·5 % (95 % CI 3·5, 10·9) in the placebo group ($P = 0·03$). 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)[1]

GDM definitions are variable, and establishing an international consensus on diagnostic criteria that predict adverse pregnancy outcomes has been challenging. In 2008, the International Association of Diabetes and Pregnancy Study Group

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



Prevalence of Gestational Diabetes

Table 2. Treatment effects on the prevalence of gestational diabetes mellitus defined according to International Association of Diabetes and Pregnancy Study Groups (IADPSG)* and New Zealand (NZ)† definitions, and mean blood glucose levels
(Prevalence percentages and 95% confidence intervals; relative rates (RR) and 95% confidence intervals; mean values and 95% confidence intervals)

	HN001		Placebo				
	Prevalence (%)	95% CI (%)	Prevalence (%)	95% CI (%)	RR	95% CI	*P*
IADPSG* (*n* 373)	8·2 (15/184)	4·6, 13·1	13·8 (26/189)	9·2, 19·5	0·59	0·32, 1·08	0·08
NZ† (*n* 394)	2·1 (4/194)	0·6, 5·2	6·5 (13/200)	3·5, 10·9	0·32	0·11, 0·96	0·03

Effect on fasting glucose levels

Table 2. Treatment effects on the prevalence of gestational diabetes mellitus defined according to International Association of Diabetes and Pregnancy Study Groups (IADPSG)* and New Zealand (NZ)† definitions, and mean blood glucose levels
(Prevalence percentages and 95% confidence intervals; relative rates (RR) and 95% confidence intervals; mean values and 95% confidence intervals)

	HN001		Placebo				
	Mean	95% CI	Mean	95% CI	Difference in mean	95% CI	*P*
Fasting (mmol/l)	*n* 195		*n* 202				
	4·32	4·27, 4·37	4·40	4·34, 4·46	−0·08	−0·15, 0·00	0·048

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 > 35years of age and in mothers with a previous history of GDM

Influence of age and previous history of GDM		Prevalence of GDM		P
		HN001™	Placebo	
Influence of age	> 35 years	7.1%	22.9%	0.009
	< 35 years	8.8%	8.5%	0.94
Previous History	With previous history	0%	87%	0.004
	No previous history	6.2%	12.3%	0.14

Wickens at al. 2017 British Journal of Nutrition

EBioMedicine 13 (2016) 190–200

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journal homepage: www.ebiomedicine.com

Research Paper

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

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Synbiotic

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 ssp. 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 (NCT01978691).

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 interven-

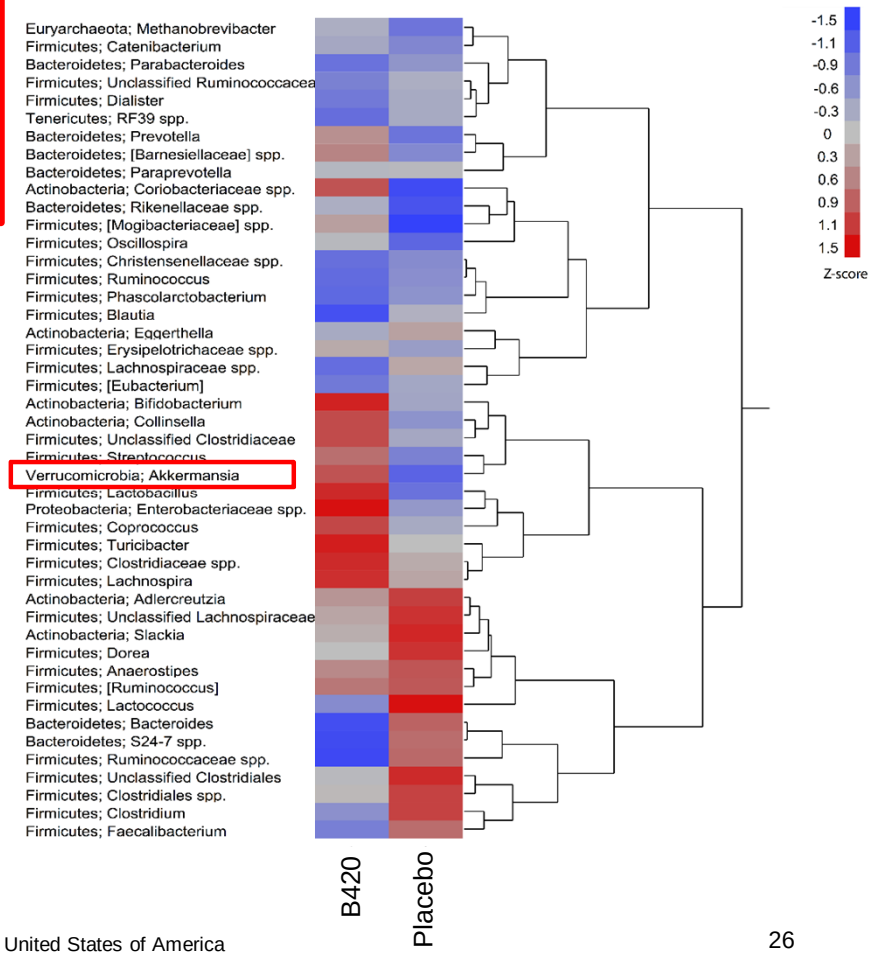
Akkermansia is more abundant in human subjects consuming B-420™

- B-420™ impacted many species of the human gut microbiota; including, significantly higher relative abundance of *Akkermansia muciniphila* in B-420™ subjects[1].

- 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[2, 3, 4].

- *A. muciniphila* has been identified as inversely correlated with body weight gain in pregnant women.[2]

- *A. muciniphila* controlled gut barrier function, fat mass storage, and glucose tolerance in obese and type 2 diabetic mice[3].

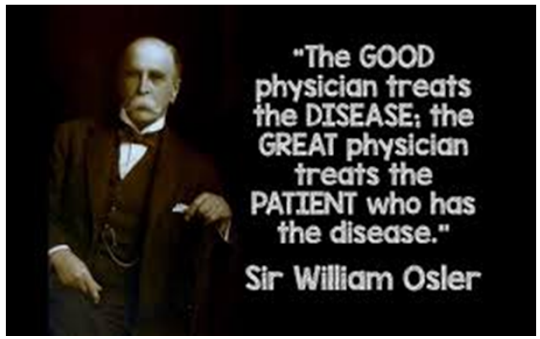


[1]Hibberd et al. 2018. Beneficial Microbes (under peer review)
[2]SantaCruz et al. 2010. British Journal of Nutrition
[3]Everard et al., 2013. Proceedings of the National Academy of Sciences of the United States of America
[4]Dao et al., 2016. Gut

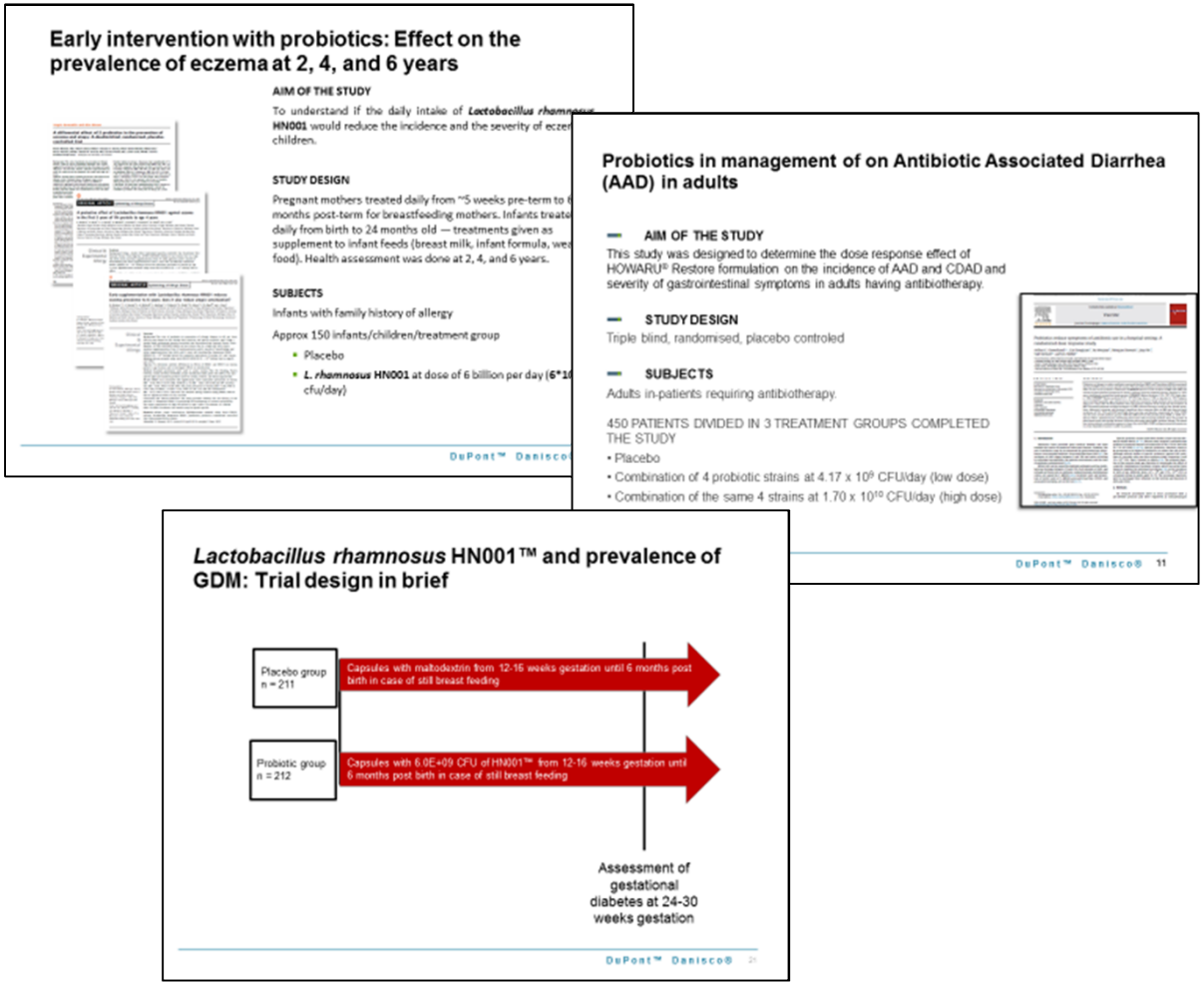
Harnessing the gut microbiome and probiotics,
for better health in a broader population

Probiotics in

Treatment



Prevention


Early intervention with probiotics: Effect on the prevalence of eczema at 2, 4, and 6 years
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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*10
cfu/day)
DuPont™ Danisco®
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.
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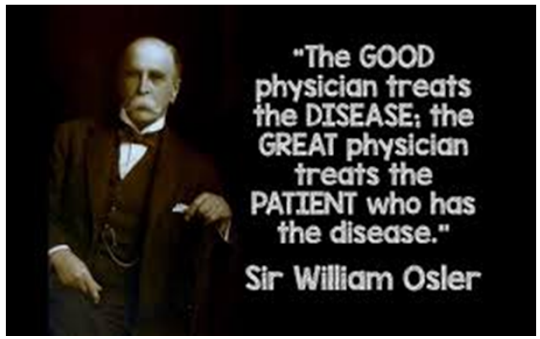
Triple blind, randomised, placebo controled
SUBJECTS
Adults in-patients requiring antibiotherapy.
450 PATIENTS DIVIDED IN 3 TREATMENT GROUPS COMPLETED THE STUDY
• Placebo
• Combination of 4 probiotic strains at 4.17 x 10^9 CFU/day (low dose)
• Combination of the same 4 strains at 1.70 x 10^10 CFU/day (high dose)
DuPont™ Danisco® 11
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
DuPont™ Danisco®

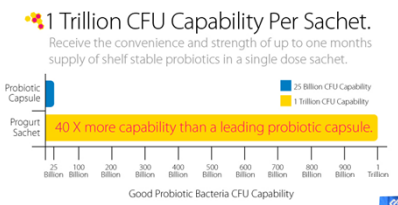
Probiotics in

Treatment



Prevention

Opportunities with different delivery formats



Product Description

KEY PRODUCT FACTS	
PER 30g SERVING	
PROTEIN	24.3g
TOTAL FATS	1.0g
CARBOHYDRATES	1.2g
KEY INCLUSIONS	
INSTANTISED WHEY PROTEIN	
MULTIVITAMIN COMPLEX	
DIGESTIVE ENZYMES	
PRE & PROBIOTICS	

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