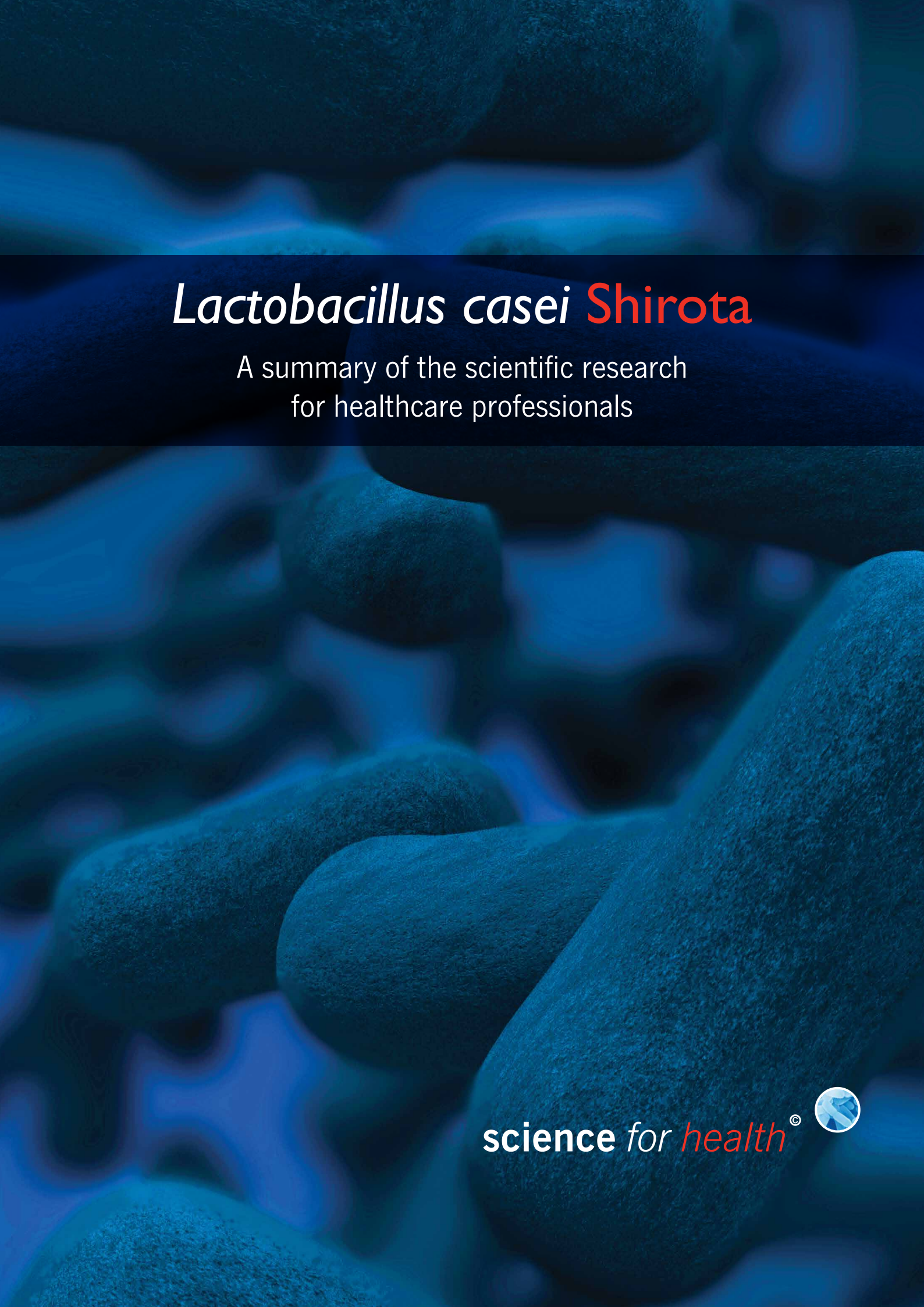

Lactobacillus casei Shirota

A summary of the scientific research
for healthcare professionals

science *for health*©

Content

Introduction

Dear Reader,

As a healthcare professional, you certainly have an interest in all approaches that may help to maintain or restore health. Today our current knowledge on the importance of nutrition for health goes much further than the fact that vitamins are essential. Over the last few decades the importance of the gut microbiota has become widely recognised. However, the ever-increasing number of diseases that have been 'associated' with a microbiota 'deficiency' has been the basis of some scepticism of the underlying science behind these observations. The complexity and individual diversity of the gut microbiota have added to this scepticism. Still the progress in our understanding of the interactions between the host and their microbiota has been fascinating. New health concepts have been discovered, and new therapeutic or prophylactic routes are ready for further exploration.

One of these routes, however, is actually not so new: the probiotic route, discussed in this booklet.

The probiotic pioneer

Lactobacillus casei Shirota, the strain unique to Yakult, was selected and cultivated in 1930 by the scientist Dr Minoru Shirota working in Japan. Inspired by Professor Elie Metchnikoff's theory that there are health benefits in replacing harmful proteolytic microbes in the colon with beneficial saccharolytic lactic acid bacteria, Dr Shirota spent years screening a collection of lactic acid bacteria to look for one with the ability to resist exposure to gastric acid and bile salts. After selecting and cultivating this strain, he used it to develop a probiotic fermented milk drink.

A company focused on science

As well as having Yakult research institutes in Japan and Belgium, the Yakult company also sponsors and supports studies by independent researchers in hospitals, universities and institutes worldwide. New papers are always being published, with over 470 papers published to date.

Furthermore, Yakult organises national and international scientific symposia on the latest scientific developments relating to probiotics, such as the International Yakult Symposium. In Europe, the company also sponsors awards for healthcare professionals and researchers (including students), and supports digestive health charities.

Lactobacillus casei Shirota research

This document contains an overview of the different research areas explored using the *Lactobacillus casei* Shirota strain, the strain found in Yakult. While research into *Lactobacillus casei* Shirota actually began in the 1930's and the strain has been consumed for over 80 years, this booklet can only present a limited selection of the more recent developments in the field of probiotics. To access original abstracts and papers mentioned throughout this booklet a reference list has been provided at the back, however you can also visit our websites that have been specifically created for healthcare professionals. Here you can access original abstracts and papers, explore further research, sign up to newsletters, download educational resources or find out about local conferences, symposia and training workshops. Your local Yakult Science Team is also available to contact should you have further queries or requests.

We hope you enjoy reading this overview,

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Probiotics - what to look for?

Definition

The term 'probiotic', first coined back in the 1950s, was formally proposed in 1965 by Lilley and Stilwell. As a result of growing scientific and commercial interest in this sector, the Food & Agriculture Organisation (FAO) and the World Health Organisation (WHO) consulted experts. The resulting working group published guidelines for the evaluation of probiotics in food, and agreed a definition for this term.

> Probiotics are defined as: '*Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host*' (FAO/WHO Working Group 2002, Hill *et al.* 2014)

Product quality

Quality assurance programmes should be in place to ensure this. For example, each batch should be tested at the beginning, during and end of shelf life to check there are sufficient numbers of viable probiotic bacteria, as stated on the product label.

Clear and sufficient information on the product

Probiotic products should state the nutritional information, the full name of the probiotic strain and the number of live probiotic cells in the product (e.g. a minimum of 6.5 billion live cells of *Lactobacillus casei* Shirota at end of shelf life).

> Full strain name = genus (e.g. *Lactobacillus*)
> + species (e.g. *casei*)
> + strain designation (e.g. Shirota).

Safety

Lactic acid bacteria have been consumed for centuries in fermented foods. With regard to probiotics, *Lactobacillus casei* Shirota (and its fermented milk product) has an unparalleled history of safe use, having been consumed by the general public for more than 85 years, as well as being used by independent researchers and clinicians for a range of patients. For further safety considerations see page 9.

Survival through the gut

This is an important characteristic for probiotics that mediate their effects via the gastrointestinal tract. For evidence of *Lactobacillus casei* Shirota survival through the gut and effect on the intestinal microbiota, see pages 5-7.

Evidence of benefit

Probiotic effects are considered to be strain-specific. Healthcare professionals should be able to find scientific evidence for products from the manufacturer. Research with *Lactobacillus casei* Shirota, for example, has shown positive health effects relating to gut health, gut function and immune modulation.

An overview of *Lactobacillus casei* Shirota research

1. Fundamental research

Fundamental research includes studies showing survival of the strain in the gut, safe consumption for humans, beneficial modulation of the intestinal microbiota and metabolites, and strain identification and stability.

1.1. Survival through the gastrointestinal tract

Survival through the gut is considered a key characteristic of probiotic strains as the mechanism of activity underlying most health benefits is associated with the transient presence, growth and activity of the live probiotic cells in the gut. The strongest evidence is detection of the strain in the faeces of people after they have consumed the probiotic.

In vitro or model studies are not proof of gut survival *in vivo*, however they do provide useful information on factors that can affect the viability of the strains. When *Lactobacillus casei* Shirota (*L. casei* Shirota) was exposed to physiological levels of gastric-, bile-, and pancreatic juices, for a realistic time of digestion, it was confirmed to have an excellent survivability, particularly when embedded in a milk matrix (Lo Curto *et al.* 2011).

The *L. casei* Shirota strain was selected because of its ability to survive the harsh conditions of the gut. There are several research papers describing human studies showing the survival of *L. casei* Shirota, encompassing a mix of population groups and a variety of ages, geographical locations and health conditions.

Example Study

Survival of *L. casei* Shirota through the gut
Spanhaak *et al*. (1998) *Eur J Clin Nutr* 52:899-907.

Method

This double-blind, placebo-controlled, randomised trial in the Netherlands involved 20 healthy adult men drinking either 3 x 100ml a day of fermented milk containing 10^9 colony forming units (CFU) *L. casei* Shirota per ml, or the same quantity of an unfermented milk placebo, for 4-weeks. Faecal samples were collected throughout the trial to assess the human gut survival of *L. casei* Shirota

Results

L. casei Shirota was detected at significantly increased levels in the faeces of subjects in the treatment group during the intervention period, compared to the placebo group ($P<0.01$). In the treatment group, *L. casei* Shirota was detected at levels reaching 10^7 cfu/g of wet faeces (see Figure 1). Once ingestion of LcS had ceased, levels decreased and reverted back to their pre-intervention state. Additionally, in only those consuming *L. casei* Shirota, there was a significant increase in *Bifidobacterium* ($P<0.05$).

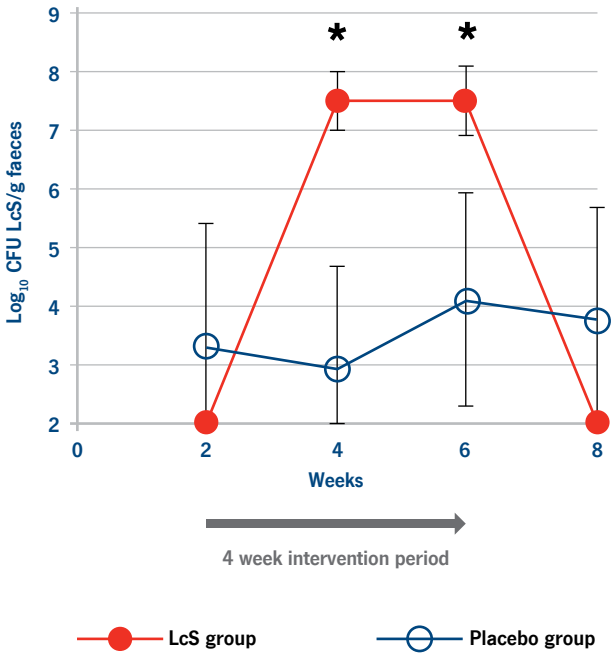


Figure 1 | Mean numbers of faecal *L. casei* Shirota (LcS) in volunteers given the probiotic.

Other examples of studies that have shown survival of *L. casei* Shirota include, but are not limited to, studies conducted in healthy adults within European populations (Tilley *et al*. 2014, Tuohy *et al*. 2007) as well as several Asian populations (Mai *et al*. 2017, Utami *et al*. 2015, Wang *et al*. 2015a), elderly populations (Bian *et al*. 2011), pre-school and school-age children (Wang *et al*. 2015b) and critically ill children (Srinivasan *et al*. 2006).

1. Fundamental research (continued)

1.2. Increase of beneficial bacterial species in the gut

Lactobacilli and bifidobacteria are generally considered to be beneficial in the gut because:

- they can produce beneficial metabolites (i.e. short chain fatty acids, monosaccharides, vitamins);
- their growth promotes acidic conditions in the colon, inhibiting harmful bacteria;
- reduced levels of bifidobacteria and/or lactobacilli are associated with certain disease states (e.g. functional gut disorders, allergy, antibiotic-associated diarrhoea);
- positive health benefits have been observed when their numbers are restored or maintained.

Lactobacillus: Gram-positive facultative anaerobic rods found widely in nature, often used to make fermented foods such as cheese, yoghurt, pickles, salami etc. Considered part of the normal commensal intestinal microbiota; detected in adults at about 10^6 to 10^8 cells per gram of faeces (wet weight).

Bifidobacterium: Gram-positive anaerobes, often with a branched (bifurcated) appearance. Produce lactic acid but generally not considered 'real' lactic acid bacteria due to phylogenetic and metabolic differences. Early colonisers of the gut; breast feeding promotes this. Major constituents of the adult colonic microbiota, detected at about 10^8 to 10^{10} cells per gram of faeces (wet weight).

The ability for *L. casei* Shirota to modulate the gut microbiota has been investigated in a number of studies (Bian *et al.* 2011, Kato-Kataoka *et al*. 2016, Motoori *et al.* 2017, Nagata *et al*. 2011, Pirker *et al.* 2012, Rao *et al.* 2009, Yamagishi *et al*. 1974). For 6-months, a group of twenty-three Japanese children consumed daily a fermented milk containing $4x10^{10}$ CFU of *L. casei* Shirota. Stool samples were collected at baseline and months (m) 1, 3, 6, and 6-months after ingestion. During the ingestion period, *L. casei* Shirota was detected at levels reaching 10^7 cells/g. Additionally, there were significant increases in total *Bifidobacterium* (3m $P<0.05$, 6m $P<0.01$) and *Lactobacillus* (1m, 3m, 6m, $P<0.01$). Six-months after the intervention, these increases had returned back to their baseline levels (Wang *et al.* 2015b).

Example Study

Increase in lactobacilli and bifidobacteria in the gut (healthy adults)
Tuohy *et al.* (2007) *J Appl Microbiol* 102(4):1026-1032.

Method

This double-blind, placebo-controlled study at the University of Reading, involved 20 healthy volunteers who for 21 days consumed either *L. casei* Shirota (at least 13×10^9 CFU) as a fermented milk drink or placebo. Stool samples were collected at days 0, 7, 14, 21, and 28 to determine survival of *L. casei* Shirota and to determine changes to the faecal bacteria.

Results

Seven days after subjects started to take the probiotic, *L. casei* Shirota was recovered at a mean level of 1.1×10^7 CFU/g of faeces, and was maintained at this level over the course of probiotic feeding, but decreased after cessation of the feeding regime. Concurrently, in subjects consuming *L. casei* Shirota, an increase in total lactobacilli was observed, which persisted even after the regime had stopped. Bifidobacteria were also found to increase during the regime, but this increase was not sustained once consumption of *L. casei* Shirota had stopped (see Figure 2).

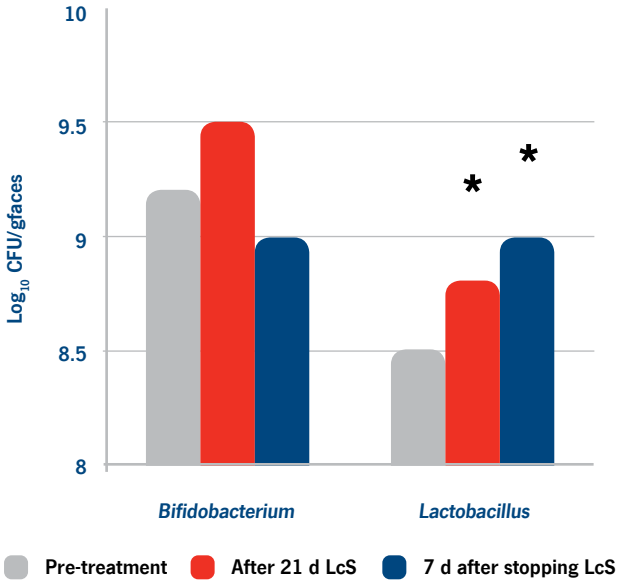


Figure 2 | Mean bacterial numbers in volunteers fed *L. casei* Shirota (LcS), determined by FISH.

1. Fundamental research (continued)

1.3. Decrease in harmful bacterial species in the gut

The intestinal microbiota comprises a range of bacteria: some beneficial, some neutral for health, and some that are pathogenic or harmful. In the latter case, this may be due to production of toxins, carcinogens or other substances that, over a period of time, may be associated with a negative effect on health.

In an early study by Dr Shirota, infants who were fed *L. casei* Shirota fermented milk for 14 days were shown to have decreased levels of Enterobacteriaceae and streptococci, compared to infants who were fed a placebo fermented milk drink (heat-treated to kill the *L. casei* Shirota) (Shirota *et al.* 1966).

Since then, several studies with *L. casei* Shirota have reported that its consumption has been associated with reduction of harmful bacterial species in the gut (Kato-Kataoka *et al.* 2016, Nagata *et al.* 2011, Nagata *et al.* 2016, Tsuji *et al.* 2014).

The faecal microbiota of female workers at Yakult in Japan, who regularly consume *L. casei* Shirota, compared to a similar cohort of women who do not regularly consume *L. casei* Shirota, showed increased levels of *Bifidobacterium* and *Lactobacillus* ($P<0.05$), and also decreased levels of *Prevotella* and *Staphylococcus* ($P<0.05$) (Tsuji *et al.* 2014).

Example Study

Reduction of potentially harmful bacteria in the gut
Nagata *et al*. (2016) *Ann Nutr Metab* 68(1):51–59.

Method

A double-blind, placebo-controlled randomised trial of residents (n=72) and staff (n=20) at a facility for the aged, consumed either an *L. casei* Shirota fermented milk drink (4×10^{10} CFU) or placebo daily for 6 months. Faecal samples were collected from all subjects at baseline and at months 1, 3 and 6.

Results

In those who had consumed *L. casei* Shirota, faecal analysis identified a significant increase in bifidobacteria, and a significant decrease in *Clostridium difficile, Clostridium perfringens*, and Enterobacteriaceae in both residents and staff. Additionally, the elderly residents who had consumed *L. casei* Shirota, had significantly lower levels of *Staphylococcus* and *Pseudomonas*.

Interestingly, residents who had consumed *L. casei* Shirota, had significantly lower incidence of diarrhea and constipation, and fewer days with a fever, compared to placebo ($P<0.05$).

1. Fundamental research (continued)

1.4. Decrease in harmful substances and improved gut milieu

There is no consensus of 'an ideal intestinal microbiota', but it is generally considered healthier for it to be predominantly saccharolytic, resulting in the production of short chain fatty acids such as butyrate, acetate, and propionate. These metabolites increase gut motility, decrease pH, provide energy for the commensal bacteria and help absorb minerals. In contrast, proteolytic fermentation results in potentially toxic and carcinogenic metabolites including ammonia, phenols, indoles and amines.

Example Study

Decrease in toxic bacterial metabolites in the gut (healthy adults)
De Preter *et al.* (2004) *Brit J Nutr* **92**:439-446.

Method

Researchers in Belgium investigated the effects of *L. casei* Shirota on toxic fermentation metabolites (NH_3 and *p*-cresol) in the gut. In this crossover study, healthy subjects (n=19) consumed either an *L. casei* Shirota fermented milk drink (6.5 x10^9 CFU), a prebiotic or the respective placebo twice a day for two weeks, with a two-week washout period in-between. A test meal was consumed at the end of weeks 2, 4, and 6, which contained stable isotope-labelled biomarkers (a ^2H and a ^{15}N marker). Urine samples were collected before each test meal, and for the following 48-hours after the meal to determine phenolic compounds, total nitrogen, and ^{15}N.

Results

The data indicated a reduction in production of the toxic fermentation metabolites NH_3 (^{15}N biomarker, P=0.047) and *p*-cresol (^2H biomarker, P=0.032) for the probiotic group, which was significantly different to the placebo group (P=0.016 and P=0.042, respectively).

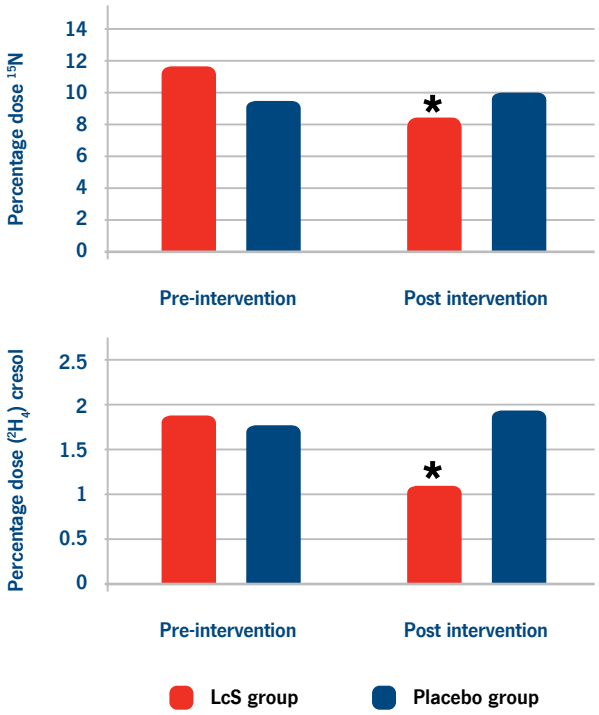


Figure 3 | Influence of *L. casei* Shirota consumption on faecal levels of ^{15}N and *p*-cresol.

Other studies have confirmed association of *L. casei* Shirota consumption with significant decrease of urinary p-cresol excretion (De Preter *et al.* 2007) and an *in vitro* study showed binding of aflatoxin by this strain (Hernandez-Mendoza *et al.* 2009).

Reducing acrylamide in foods

Acrylamide is a potential carcinogen found in some foods, and an *in vitro* model assessing the ability of 14 *Lactobacillus* strains to bind to acrylamide found *L. casei* Shirota had one of the highest binding abilities. At concentrations of 5µg/ml, *L. casei* Shirota was able to remove up to 24.95% of acrylamide (Serrano-Niño *et al.* 2014). Furthermore, in a dynamic model system simulating the gastrointestinal tract, researchers found that *L. casei* Shirota removed 65-73% of acrylamide in potato chips – a food with low acrylamide content. Repeating this experiment using food with originally high acrylamide content led to a 5-10% removal rate of acrylamide, which could be due to a possible saturation of binding sites of the bacteria after exposure to a certain acrylamide concentration (Rivas-Jimenez *et al.* 2016).

1. Fundamental research (continued)

1.5. Safety

Lactic acid bacteria have been consumed for centuries in fermented foods. With regard to probiotics, *L. casei* Shirota (and its fermented milk product) has an unparalleled history of safe use, having been consumed by the general public on a very wide scale for more than 85 years, as well as being used by independent researchers and clinicians across a range of patients. Across all of the studies published to date, there have been no reports of serious side effects or adverse events. Similarly, there has never been any report on the uptake of transferrable antibiotic resistance genes by *L. casei* Shirota.

Critically Ill Patients

The *L. casei* Shirota strain has been used in clinical trials conducted in seriously ill patients with conditions such as alcoholic cirrhosis (Stadlbauer *et al.* 2008), severe systemic inflammatory response (Shimizu *et al.* 2009), on long-term mechanical ventilation (Hayakawa *et al.* 2012), elective liver donor patients (Eguchi *et al.* 2010), bladder, biliary and colorectal cancer patients (Kanazawa *et al.* 2005, Naito *et al.* 2008, Sugawara *et al.* 2006), very-low birth weight preterm infants (Braga *et al.* 2011) and critically ill children in intensive care (Srinivasan *et al.* 2006) as detailed further. Case reports have also reported the use of *L. casei* Shirota in patients with severe respiratory distress (Kanamori *et al.* 2006b) and short bowel syndrome (Candy *et al.* 2001, Kanamori *et al.* 2001, Uchida *et al.* 2007).

Enteral Nutrition Support

There are also clinical trials and case reports on delivery of *L. casei* Shirota via enteral feeding tubes including nasogastric (NG), nasojejunal (NJ), and gastrostomy (Kanamori *et al.* 2010, Kanazawa *et al.* 2005, Shimizu *et al.* 2009, Srinivasan *et al.* 2006, Sugawara *et al.* 2006). See considerations and further reading below if you are considering using probiotics in enteral feeding regimes.

The study detailed here is a safety study – a study designed with the primary objective of assessing safety - conducted using *L. casei* Shirota delivered via enteral feeding tubes in critically ill children.

Example Study

Clinical safety in critically ill children
Srinivasan *et al.* (2006) *J Ped Gastroenterol Nutr* 42:171-173.

Method

The objective of this study was to establish clinical safety of *L. casei* Shirota used as a probiotic in critically ill children. *L. casei* Shirota was administered three times a day at a dosage of 10^7 CFU/day via an indwelling nasogastric tube for five days to children admitted to a paediatric intensive care unit in the UK. Safety was assessed by bacteriologic surveillance for the strain in surface swabs, endotracheal aspirates and blood, urine and sterile body fluid samples.

Results

From the 28 patients with available safety data, there was no evidence of either colonisation or bacteraemia with *L. casei* Shirota from this testing. The *L. casei* Shirota was well tolerated with no apparent side effects or adverse reactions, supporting the conclusion that *L. casei* Shirota as a probiotic in critically ill children fed via a nasogastric tube appears safe.

Considerations

Although many of the case reports, intervention studies and meta-analyses that describe probiotic use in seriously ill patients report evidence of benefit, most also caution that more research is needed.

If considering administration of a high dose of probiotics via a nasojejunal tube, alongside enteral nutrition containing a high concentration of fermentable sugars, utmost caution and close monitoring should be undertaken. This is especially important in patients with non-occlusive mesenteric ischemia, common in critically ill patients such as acute pancreatitis patients for whom this regime would not be advised (Besselink *et al*. 2008).

When considering to use probiotics for a specific patient where you may have safety concerns, it is important to consider (i) the quality and safety record of the particular probiotic; (ii) the administration mode and (iii) the patient's condition.

Recommended further reading

Whelan K & Myers CE (2010) Safety of probiotics in patients receiving nutritional support: a systematic review of case reports, randomized controlled trials, and nonrandomized trials. *Am J Clin Nutr* **91**:687-703.
Hempel S *et al.* (2011) Safety of probiotics to reduce risk and prevent or treat disease. Evidence Report/Technology Assessment No. 200. (Prepared by the Southern California Evidence-based Practice Center under Contract No. 290-2007-10062-I.) AHRQ Publication No. 11-E007. Rockville, MD: Agency for Healthcare Research and Quality. Available at: **www.ahrq.gov/research/findings/evidence-based-reports/probiotsum.html**

2. Functional gut disorders

A range of human trials have investigated *L. casei* Shirota effects on functional gut disorders including irritable bowel syndrome, constipation, diarrhoea and inflammatory bowel disease.

2.1. Irritable bowel syndrome

Irritable bowel syndrome (IBS) is one of the most common gastrointestinal disorders, affecting approximately 10% of the population worldwide, and about 15% of the Western population. Women are affected more frequently, in particular young women under 50 years of age (Lovell & Ford 2012). IBS can be a debilitating condition, severely affecting quality of life, comparable to the quality of life of someone with diabetes or depression (Suares *et al.* 2011). The symptoms of IBS are diverse and the exact causes remain unclear, but in most cases they are multifactorial. Many studies have associated changes in the intestinal microbiota with IBS pathophysiology (reviewed by Rajilic-Stojanovic *et al.* 2015), often characterised by:

- Reduced diversity of intestinal bacteria
- Increased relative proportion of Firmicutes, especially *Clostridium* cluster XIVa and Ruminococcaceae.
- Decreased relative proportion of Bacteroidetes.

In addition, there is an increased risk of developing IBS after bacterial gastroenteritis or antibiotic therapy, further substantiating the gut microbiota's involvement in IBS.

A recent meta-analysis shows that probiotics can be effective in IBS. In particular, single strain probiotics and a short treatment period improved overall symptoms and the quality of life more effectively than placebo (Zhang *et al.* 2016). The growing body of evidence has led to guidelines advising that should a patient choose to try a probiotic product they should take the probiotic for at least four weeks and monitor symptoms (Layer *at al.* 2011, McKenzie *et al*. 2016, NICE Clinical Guideline CG61 2008).

In a pilot study examining the effects of *L. casei* Shirota on small intestinal bacterial overgrowth (SIBO) in patients with IBS, the researchers found the intervention to be effective in altering fermentation patterns consistent with reducing SIBO (Barrett *et al.* 2008).

2.2. Constipation and transit time

Constipation is usually a disorder of the motor activity of the colon. The importance of the intestinal microbiota for bowel habit and gut function is highlighted by alterations in subjects with constipation, e.g. lower levels of lactobacilli and bifidobacteria (Khalif *et al.* 2005). The composition of the microbiota is also associated with motility of the gastrointestinal tract (Barbara *et al.* 2005), for which transit time is a useful measure. Microbial production of short chain fatty acids and metabolism of bile acids is important for lowering gut pH and stimulating motility.

Reviewing more than 21 studies with 2,656 constipated subjects displayed a benefit of probiotics: stool frequency and intestinal transit time was significantly improved by supplementation with lactobacilli or bifidobacteria (Miller *et al.* 2017).

Several studies exploring effects of *L. casei* Shirota on constipation and transit time have been conducted, including human trials and mechanistic studies (Aoki *et al.* 2014, Cassani *et al.* 2011, Koebnick *et al.* 2003, Krammer *et al.* 2011, Mazlyn *et al.* 2013, Sakai *et al.* 2011, Sakai *et al.* 2015, Tsuji *et al.* 2014).

Example Study

Effect on constipation symptoms (adults)
Koebnick *et al.* (2003) *Can J Gastroenterol* 17:655-659.

Method

In this double-blind, placebo-controlled, randomised trial conducted in Germany, 70 chronically constipated adults drank one bottle a day of probiotic fermented milk containing *L.casei* Shirota (6.5 x10^9 CFU) or placebo for four weeks. Efficacy was assessed by a weekly medical examination and patient questionnaire (gastrointestinal symptoms; wellbeing; bowel habit). The severity of constipation, flatulence and bloating was graded as severe, moderately severe, mild, or no symptoms.

Results

Starting from the second week of drinking, a significant improvement of certain constipation symptoms was observed for those in the probiotic group (all P<0.001).

Parameter	Placebo	Probiotic	*P*
Occurrence of moderate-severe constipation	83%	34%	<0.001
Occurrence of hard stools	82%	29%	<0.001
Stool consistency (5 point scale, modified Bristol Stool Scale)	5	3	<0.001

Table 1 | Constipation occurrence and stool consistency post-intervention with *L. casei* Shirota

Example Study

Effects on transit time
Krammer *et al.* (2011) *Coloproctology* 33:109-113.

Methods

In this double-blind, placebo-controlled trial, 24 patients with chronic constipation (transit time >72h) received a drink containing *L. casei* Shirota (6.5 x 10^9 CFU) or placebo, daily for 4-weeks. A Hinton Test with radiopaque markers was performed on all participants at baseline and after the intervention to assess the colonic transit time. General gastrointestinal symptoms were recorded using a weekly questionnaire.

Results

L. casei Shirota led to a significant acceleration of the colonic transit time from 95.6 to 76.5 hours (P=0.05). This effect was most marked in the sigmoid and rectal transit times (P<0.007). The change in transit time from 98.8 to 87.1 hours in the placebo group was not statistically significant (P=0.282).

Conclusion

L. casei Shirota can shorten the colonic transit time in patients with slow transit constipation.

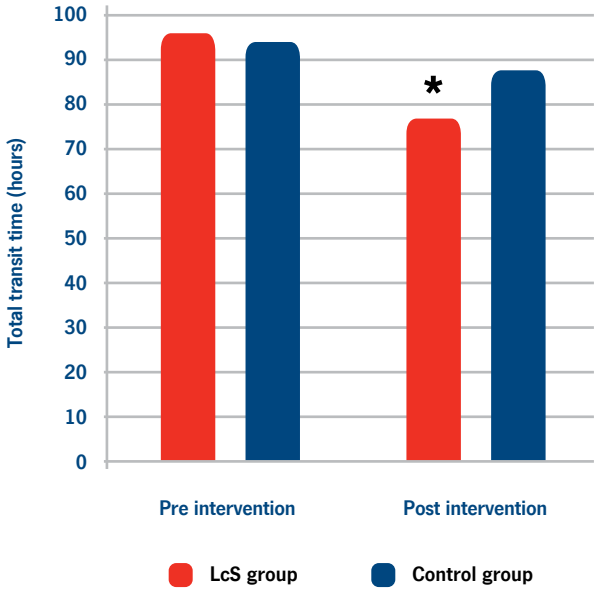


Figure 4 | Effect of *L. casei* Shirota consumption on transit time

2.3. Stool consistency

Besides transit time and the frequency of bowel movements, stool consistency is an important part of healthy bowel habits. Stool consistency is mainly determined by the faecal water content. Harder and dryer stools are often perceived as gastrointestinal discomfort without necessarily being associated with constipation or IBS.

Interestingly, stool consistency strongly associates with known microbiome markers. For example, microbial diversity in the gut decreases with higher Bristol Stool Scale scores of 6-7 (see Figure 5), indicating a more liquid stool. Furthermore, the different enterotypes are distinctly assigned to Bristol Stool Scale ranges (Vandeputte *et al.* 2016).

Studies with *L. casei* Shirota have investigated human bowel habits including parameters like stool frequency, transit time and stool consistency (Krammer *et al.* 2011, Sakai *et al.* 2011, Tilley *et al.* 2014).

Improved bowel habits in the elderly

In a study conducted in 135 elderly nursing home residents in the Netherlands, consumption of *L. casei* Shirota for six weeks resulted in a significantly lower percentage of constipation (P<0.01) and diarrhoea (P=0.016), and a significant increase in the percentage of ideal stool types (P<0.01) suggesting that *L. casei* Shirota can improve the bowel habits of elderly nursing home residents (van den Nieuwboer *et al.* 2015)

Example Study

Effect on stool consistency
Tilley *et al.* (2014) *Int J Probiotics Prebiotics* **9**(1/2):23-30.

Methods

In this double-blind, placebo-controlled randomised study, 120 adults suffering from mild constipation (defined as four or less complete bowel movements per week) were assigned a daily drink with *L. casei* Shirota (6.5 x10^9 CFU) or placebo, for 4-weeks. Stool consistency was analysed using a patient questionnaire based on the visual Bristol Stool Scale.

Results

Compared to placebo, subjects taking *L. casei* Shirota experienced a significant improvement in stool consistency; the stools became softer. Both groups showed an improvement in defaecation frequency.

Type 1		Seperate hard lumps, like nuts (hard to pass).
Type 2		Sausage-shaped but lumpy.
Type 3		like a sausage but with cracks on its surface.
Type 4		like a sausage or snake, smooth and soft.
Type 5		Soft blobs with clear cut edges (passed easily).
Type 6		Fluffy pieces with ragged edges, a mushy stool.
Type 7		Watery, no solid pieces (entirely liquid).

Figure 5 | Bristol Stool Scale (from O'Donnell *et al.* 1990)

2. Functional gut disorders (continued)

2.4. Inflammatory bowel disease

The prevalence of inflammatory bowel disease (IBD) exceeds 0.3% in many European countries, with ulcerative colitis reported in 505 per 100,000 in Norway and Crohn's disease reported in 322 per 100,000 in Germany (Ng *et al.* 2017). The range of IBD conditions includes:

- **Ulcerative colitis (UC):** Onset often between the ages of 15 and 30 years. Acute and chronic inflammation affects the mucosa of all or part of the colon (always the rectum), which may become ulcerated. This commonly results in symptoms such as diarrhoea, bleeding from the anus and abdominal pain. Sufferers may also experience flatulence, constipation, passing of mucus and tiredness.
- **Crohn's disease (CD):** Any part of the gut can be affected, from mouth to anus, but inflammation is most common in the ileum and colon, and can be transmural. Symptoms are non-specific abdominal pain and diarrhoea, often with blood and mucus.
- **Pouchitis:** Affects patients with serious UC who have undergone surgical resection of the colon. The bowel is reconnected and an internal pouch created from the small intestine to hold waste before elimination. Inflammation of the lining of this pouch occurs in up to 50% of patients. Symptoms include abdominal pain, more frequent bowel activity and rectal bleeding, as well as malaise and fever.

Different lines of evidence suggest the intestinal microbiota may be involved in IBD pathogenesis, suggesting there may be benefit in manipulating the intestinal microbiota, perhaps with probiotics (Derwa *et al.* 2017).

- Antibiotic use is associated with the occurrence of CD
- The diversity of gut bacteria is reduced in in IBD patients
- Patients with IBD have been shown to have an altered microbiome compared to healthy subjects
- Anti-inflammatory species, e.g. *Faecalibacterium prausnitzii,* are reduced in IBD, while pro-inflammatory species are more abundant
- During active phases of IBD the gut microbiota changes

In terms of probiotic research and IBD, there is limited evidence of benefit. A recent meta-analysis states that "probiotics might be as effective as 5-ASA in preventing relapse of quiescent UC" (Derwa *et al.* 2017). Research using *L. casei* Shirota has been conducted in patients with active CD (Fujimori *et al.* 2007) and UC (detailed below), and mechanistic studies have been conducted using cells from patients with UC (Mann *et al.* 2011, Mann *et al.* 2013, Mann *et al.* 2014).

Example Study

Effects on symptoms of ulcerative colitis (adults)
Mitsuyama *et al.* (2008) *J Clin Biochem Nutr* **43**(Suppl 1):78-81.

Method

In an open-label trial, ten patients with mild to moderately active UC consumed a daily fermented milk drink containing *L. casei* Shirota (8 x 10^{10} CFU) for eight weeks in conjunction with conventional therapy (aminosalicylates and/or prednisolone). Changes in clinical status were measured by a clinical activity index score at baseline and at two-week intervals, which recorded several disease aspects (diarrhoea episodes, nocturnal diarrhoea, visible faecal blood, abdominal pain or cramping, general wellbeing, abdominal tenderness, need for anti-diarrhoeal medication). The control group were nine previously-treated patients with active UC, whose baseline characteristics were similar to the study group and who had previously received conventional therapy but not a probiotic.

Results

Compared to the control group, probiotic consumption was associated with significantly improved clinical activity index scores after four weeks (P=0.033), six weeks (P=0.026) and eight weeks (P=0.012). When compared to pre-treatment clinical activity index scores, a trend for improved clinical status was observed in the probiotic group but not in the control group, at six weeks (P=0.010) and eight weeks (P=0.035). There were indications that the mechanism of activity may involve inhibition of IL-6 signalling.

3. Infectious disease

Medical and scientific experts acknowledge the importance of the commensal intestinal microbiota in the body's defense against infection. The intestinal microbial population is important for maintaining colonisation resistance in the gut, as well as for educating and supporting the immune system.

3.1. Gut-related infections

Since their first development, there has been interest in using probiotics against gut infections, particularly diarrhoeal illness. Cochrane reviews in 2010 concluded that, used alongside rehydration therapy, probiotics are safe and have clear benefits in shortening duration and reducing stool frequency in acute infectious diarrhoea, although the data for children was limited (Allen *et al.* 2010, Bernaola Aponte *et al.* 2010).

Research with *L. casei* Shirota has investigated diarrhoea of undetermined aetiology, as well as infection with a range of pathogens, either bacterial (*Clostridium difficile* (Lewis *et al.* 2009, Martinez *et al.* 2003, Pirker *et al.* 2012), *Helicobacter pylori* (Cats *et al.* 2003, Sgouras *et al.* 2004)) or viral (rotavirus (Jacalne *et al.* 1990), norovirus (Nagata *et al.* 2011)). Subjects in trials have included children, adults and older patients in hospitals or care homes. Supporting evidence also comes from extensive mechanistic and model studies.

3.1.1. Antibiotic-associated diarrhoea & *Clostridium difficile* infection

Diarrhoea is a common side effect of antibiotic treatment, occurring in 5 - 39% of people taking antibiotics or within 2-3 weeks of finishing a course (McFarland 1998). Risk factors include older age, frailty, underlying morbidity and broad-spectrum antibiotics (particularly clindamycin, second- and third-generation cephalosporins, quinolones, co-amoxiclav and aminopenicillins). Around 20-30% of antibiotic-associated diarrhoea (ADD) cases are caused by *Clostridium difficile* (NICE Evidence Summary [ESMPB1] 2015). Its growth is usually prevented by the normal gut bacteria but if antibiotics disrupt this microbiota, *C. difficile* can grow unchecked, producing toxins that cause illness, ranging from diarrhoea to pseudomembranous colitis (a potentially fatal disease). Those most at risk are older hospital patients, with over 80% of reported *C. difficile* cases occurring in those over 65 years. Illness can be recurrent and fatal.

C. difficile spores can survive for long periods in the environment so will spread easily on the hands of anyone who has picked them up, perhaps from infected patients or surfaces. Good hygiene as well as strict antibiotic prescribing regimes (although limited) are the best way to prevent this. Novel non-antibiotic therapeutics for the treatment of *C. difficile* associated diarrhea are also being sought, to both eliminate the pathogen and restore the gut microbiota (Rineh *et al.* 2014).

Probiotics are known to address gut dysbiosis, one of the underlying risks for *C. difficile*, thus there has been considerable research in this area, particularly into whether risk can be reduced by taking probiotics before, during and/or after antibiotics. A Cochrane Review concluded that there is moderate certainty that "probiotics are effective for preventing *C. difficile* associated diarrhoea" in adults and children (Goldenburg *et al.* 2017). Studies have shown positive effects of the probiotic *L. casei* Shirota in reducing the incidence of *C. difficile* associated diarrhoea and lowering the risk of relapse (Lewis *et al.* 2009, Martinez *et al.* 2003, Stockenhuber *et al.* 2008, Weir 2009, Wong *et al.* 2015), examples of which are described here.

Example Study

Effects on incidence of antibiotic-associated diarrhoea and *Clostridium difficile* infection (older adult patients)
Pirker *et al.* (2012) *Food & Agric Immunol* **24**(3):315-330.

Method

This Austrian study involved 678 hospital patients (mean age 71) who were on a range of single or multiple antibiotic regimes. One group (n=340) consumed a daily fermented milk drink containing *L. casei* Shirota (6.5 x 10^9 CFU) during antibiotic treatment and for a further three days after this ceased. A control group of patients (n=338) received only their antibiotic regime.

Results

A significant reduction in both antibiotic-associated diarrhoea and *C. difficile*-associated infection was observed in those consuming *L. casei* Shirota alongside their antibiotic regime compared to control (antibiotic regime only) (Figure 6). Faecal analysis showed that antibiotic therapy alone decreased the abundance of total bacteria, *Bifidobacterium* species and *Clostridium* clusters IV and XI, with a concurrent increase in Enterobacteriaceae. However, administration of *L. casei* Shirota reduced the observed antibiotic-induced dysbiosis, and increased levels of *Lactobacillus*.

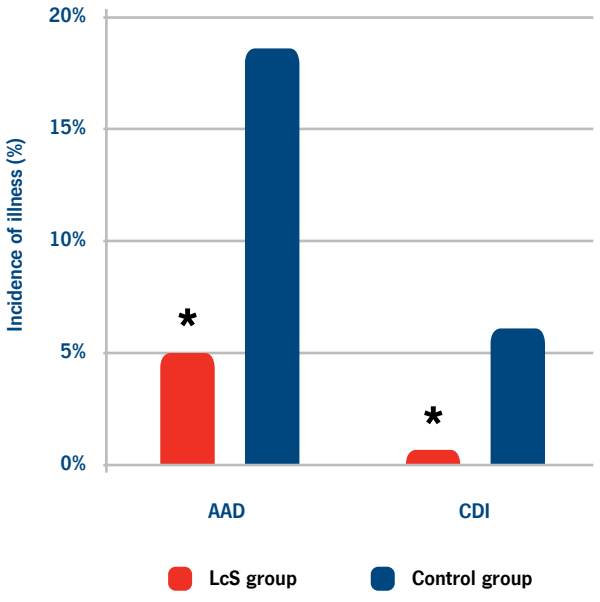


Figure 6 | Effect of *L. casei* Shirota on incidence of antibiotic-associated diarrhoea (AAD) and *Clostridium difficile* infection (CDI) in elderly hospital patients (n=678).

3. Infectious disease (continued)

3.1.1. (Continued)

Example Study

Example Study
Risk of relapse following *C. difficile* infection
Lee *et al.* (2013) *Int J Probiotics Prebiotics* **8**(3/4):145-148

Recurrence rates are high in patients who have had *C. difficile* infection (CDI): up to 35% of patients can have a relapse. The cost of treating such patients is high, estimated as up to £11,000 per episode.

Method

This single-site, cohort-controlled study in a UK hospital followed up 66 patients (median age 78 years) with CDI who, when first diagnosed, were treated with antibiotics alone or with antibiotics in combination with an *L. casei* Shirota fermented milk drink.

Results

Rates of recurrent CDI were significantly lower in the *L. casei* Shirota cohort (3.2%) compared to the control (20.0%; P=0.007). Only six patients (19.4%) in the probiotic group required re-admission for diarrhoea within three months, compared to 13 patients (35.1%) in the control group.

Example Study

Risk of antibiotic-associated diarrhoea (AAD) in spinal cord injury (SCI) patients
Wong *et al.* (2013) *Brit J Nutr* **111**(4):672-8.

Method

This randomised controlled trial at the National Spinal Injuries Centre at Stoke Mandeville Hospital, involved 164 subjects with a SCI who were on antibiotics. Subjects consumed a fermented milk drink containing *L. casei* Shirota for the duration of their antibiotic course and for 7 days thereafter, or were considered controls (no probiotic, antibiotic regime only). Bowel movements were monitored by nursing staff for the presence of AAD, and where necessary stool samples were sent to the microbiology laboratory for the detection of *C. difficile* toxin.

Results

The probiotic group had a significantly lower incidence of AAD (17.1% vs 54.9%, P<0.001). Only one patient had confirmed CDI. This patient was in the control group, i.e. not taking the probiotic.

3.1.2. Paediatric diarrhoea

Enteric infections affect many infants and young children, particularly in developing countries but even in the European countries. Almost all infants suffer at least one episode of rotavirus before they are five (Centers for Disease Control & Prevention 2008). Many studies have shown probiotic benefit in this area, either for reducing the duration of diarrhoea or risk of illness. The following study investigates whether diarrhoeal risk could be reduced with an intervention using *L. casei* Shirota.

Example Study

Effect on incidence of acute diarrhoea (children)
Sur *et al.* (2010) *Epidemiol Infect* **139**(6):919-26.

Method

This double-blind, placebo-controlled, randomised study involved 3,758 children aged one to five years, living in an urban slum in India. Every day for 12 weeks, the children drank a probiotic fermented milk drink (*L. casei* Shirota, minimum 6.5×10^9 CFU) or a placebo drink. The effects of these interventions were assessed during the drinking period and for a further 12 weeks.

Results

After 24 weeks, the incidence of diarrhoea in the probiotic group was 608 (i.e. 0.88 cases/child per year) compared to 674 for the placebo group (1.029 cases/child per year). This was equivalent to a reduction of risk of diarrhoea associated with probiotic of 14% (95% CI 4-23; P<0.01 in adjusted model).

Microbial analysis found a range of faecal pathogens, making it difficult for probiotic effect to be attributed to any particular aetiology.

3. Infectious disease (continued)

3.1.3. Norovirus

Norovirus (also known as Norwark virus or small round structured viruses) is aptly called the winter vomiting disease because of its symptoms (vomiting and diarrhoea) and its seasonality, although it can strike any time of the year. It is highly contagious and outbreaks have occurred in hospitals, nursing homes, schools and cruise ships.

Although the illness can be quite unpleasant, it should only last a few days. Advice is to rest, keep hydrated and try not to infect anyone else. Prevention is better than cure so good hygiene is important. Although there is very little probiotic research on norovirus, a trial has been conducted with *L. casei* Shirota.

Example Study

Effect on illness associated with norovirus gastroenteritis (elderly residents)
Nagata *et al.* (2011) *Brit J Nutr* **106**(4):549-56.

Method

This open label study of seventy-seven subjects (mean age 84 years) was conducted in a residential home in Japan during winter. The intervention was an *L. casei* Shirota fermented milk drink given to 39 residents; a control group of 38 did not receive the probiotic. Efficacy was assessed by looking at records of illness for the month of December when there were norovirus cases.

Results

The mean duration of fever >37 °C after onset of illness was 1.5 days (SD 1.7) in the probiotic group compared to 2.9 days (SD 2.3) in the control group, which was a significant reduction ($P<0.05$). There was, however, no significant difference in incidence of norovirus gastroenteritis during this month.

Faecal analysis of a sub-group of subjects found a significant increase in numbers of *Bifidobacterium* ($P<0.05$) and *Lactobacillus* ($P<0.01$) after one month on the probiotic. After two months there was a significant decrease in levels of Enterobacteriaceae and *Pseudomonas* ($P<0.05$).

3.1.4 *Helicobacter pylori*

Across Europe, it is estimated that between 17-88% of people are infected with *H. pylori*, with prevalence increasing with age and at its highest amongst people aged 50 years onwards (Roberts *et al.* 2016). This Gram-negative helical bacterium can colonise the gastric mucosal surface and is often picked up in childhood from other people; it then remains in the stomach unless particular antibiotics and an anti-acid are given. In the majority of cases (nearly 90%) infection does not cause any problems, however *H. pylori* is considered a pathogen because it can cause chronic inflammation of the inner stomach lining and has been linked to development of stomach cancer. It is the most common cause of ulcers: about 15% of infected people go on to develop ulcers in the stomach or duodenum.

Normal treatment is with a combination of antibiotics and an anti-acid, and there has been research investigating the benefit of probiotics either with or without these antibiotics. A meta-analysis by Zou *et al.* (2009), concluded supplementation with lactobacilli could be effective in increasing eradication rates of anti-*H. pylori* therapy for patients treated for the first time, and also had a positive effect on some of the therapy-related side effects. Studies have been conducted with *L. casei* Shirota, examining both its effects alone or in combination with antibiotic therapy (Sahagún-Flores *et al.* 2007, Sgouras *et al.* 2004).

Example Study

Effect on *Helicobacter pylori* infection in combination with triple treatment (adults)
Sahagún-Flores *et al.* (2007) *Cirugia & Cirujanos* **75**(5):333-336

Method

A randomised comparative trial involving 64 patients investigated whether there was any benefit in including the probiotic *L. casei* Shirota with conventional triple treatment (clarithromycin, amoxicillin and omeprazole) to eradicate *H. pylori* infection

Results

Based on breath test measurements, eradication of *H. pylori* was achieved in 29/31 of subjects given the probiotic (94%) compared to 25/33 of the control group (76%) ($P<0.05$).

3. Infectious disease (continued)

3.2. Other infections

Due to their ability to influence the body via multiple mechanisms of activity, probiotic effects can be evident beyond the gut. The primary mechanisms by which probiotics have effects against extra-intestinal infections, are probably via modulation and support of the immune response. Human studies have shown positive results with *L. casei* Shirota in terms of reducing upper respiratory tract infections in healthy office workers (Shida *et al.* 2017) and athletes (Gleeson *et al.* 2011), HTLV-1 myelopathy (Matsuzaki *et al.* 2005), human papillomavirus infection (linked to development of cervical cancer) (Verhoeven *et al.* 2012), post-operative infections (Eguchi *et al.* 2010, Kanamori *et al.* 2006a, Usami *et al.* 2011) and infections in elderly residents of a nursing home (Fujita *et al.* 2013).

3.2.1 Upper Respiratory Tract Infections

Upper respiratory tract infections (URTI), such as tonsillitis, laryngitis, sinusitis, flu and the common cold, are caused by an acute infection, and in 2015 there were 17.2 billion cases (Vos *et al.* 2016). A Cochrane review concluded that probiotics were more effective than placebo in reducing the number of participants experiencing an acute URTI episode, and the mean duration of an acute URTI episode (Hao *et al.* 2015). Studies using *L. casei* Shirota have been conducted in athletes and healthy office workers, as detailed here.

Example Study

Upper respiratory tract infections in healthy office workers.
Shida *et al.* (2017) *Eur J Nutr* **56**(1):45-53.

Methods

In this randomised controlled trial, 96 healthy male office workers (aged 30-49 years) were randomised to receive either a fermented milk drink containing *L. casei* Shirota (1.0×10^{11} CFU) or a control milk daily for 12 weeks during the winter months. Saliva and blood samples were taken at 0, 6 and 12-weeks, and participants also kept a daily symptoms diary.

Results

Over the intervention period, URTI rates overall were significantly lower in the probiotic group compared to the control group (22.4 vs. 53.2%, P=0.002). Furthermore, in the probiotic group, subjects had significantly lower cumulative number of URTI episodes (P=0.004) and cumulative days with URTI symptoms (P=0.001) per person, and the duration per episode was also shorter (P=0.002).

Furthermore, laboratory analysis showed that *L. casei* Shirota inhibited both the reduction in NK cell activity and increases in salivary cortisol levels that were observed in the control group, suggesting an immunomodulatory mechanism.

Incidence over the 12-wk intervention	LcS (n=49)	Control (n=47)	P-value
Upper respiratory tract infections	22.4%	53.2%	0.002
Common cold episodes	18.4%	44.7%	0.005
Influenza episodes	4.1%	4.1%	0.201

Table 2 | Effect of *L. casei* Shirota on URTI in healthy middle-aged workers.

Athletes and sports people, particularly the elite and endurance athletes, have an increased risk of infection due to their increased exposure to pathogens, and the effects on their immune system from their lifestyle and activity e.g. physiological, psychological and/or environmental stress, poor diet and/or sleep (Gleeson *et al*. 2004).

A randomised, placebo-controlled trial conducted in the UK found that regular ingestion of *L. casei* Shirota over the winter period appeared to reduce the mean number of upper respiratory tract infections in athletes, compared to placebo (1.2±1.0 vs. 2.1±1.2, P<0.01) and this was attributed to the maintenance of salivary IgA levels (an indicator of mucosal immune status) in the probiotic group (Gleeson *et al*. 2011) (see page 19).

3. Infectious disease (continued)

3.2.2 Multi-drug resistant pathogens

The emergence of antimicrobial resistance is a potential threat to treating infections in the future. Multi-resistant Gram-negative bacilli (MRGNB) are an important cause of both community-acquired and nosocomial infection, and faecal carriage of MRGNB is generally increasing (Ho *et al.* 2010, Jenkinson *et al.* 2011). Metallo-β-lactamase (MBL) strains, which hydrolyse carbapenems, have been responsible for several outbreaks worldwide (Crespo *et al.* 2004).

Example case report

Burns patient with multi-drug resistant *Pseudomonas aeruginosa*
Thomson *et al.* (2012) *J Wound Care* **21**(11): 566-569.

The majority of fatalities in burns patients are due to infected wounds, thus the spread of multi-drug resistant bacteria in hospitals is a major concern.

This case report describes a patient with serious burns that became infected with an extremely drug resistant MBL *P. aeruginosa,* which appeared to be contributing to wound breakdown and failure to heal. There was evidence that the strain had colonised the patient's gastrointestinal tract, which then acted as a reservoir for re-infection.

A dietitian suggested commencing oral treatment with an *L. casei* Shirota fermented milk drink in order to modulate the gut microbiota and suppress the pathogen.

Two weeks after the probiotic regime was initiated, the strain was no longer detected. This change persisted until the patient was discharged (15 months after first suffering the burns).

4. Immune mechanisms

While some functionalities of probiotics are considered more generic, the effect on the immune system is considered to be strain specific (Hill *et al.* 2014). Moreover, the study of immunological effects *in vivo* is difficult as currently no specific biomarkers for immune functionality have been validated. This is partly related to the fact that the immune status of each individual subject can differ considerably, based on lifestyle, health status and genetics, and, moreover, may change over time, e.g. related to stress, age or seasonal variation. Therefore it is important to collect as much information as possible from different situations, using a broad portfolio of *in vitro* and *in vivo* tests, yielding indications at the molecular, cellular and biological levels of how a specific probiotic strain, such as *L. casei* Shirota, can influence immune responses (Dong *et al.* 2009, Kaji *et al.* 2010, Kobayashi *et al.* 2010).

If the immune response does not function at maximum efficiency, susceptibility to common infectious diseases will increase. For example, it is well known that the immune response weakens in later life, increasing disease risk (Gomez *et al.* 2008), and that chronic illness also has a negative effect on the immune system. Furthermore, both psychological (Cohen & Williamson 1991) and physical stress (Gleeson 2007) may cause transient immune depression, linked to increased episodes of the common cold.

When discussing immune effects, the understanding of the mechanisms involved is extremely important as this will help to understand how probiotics can support people that are already healthy. This concept, by definition, is difficult to prove as very large-scale preventive studies need to be executed over an extensive period of time. Therefore, developing mechanistic studies might be one of the few options to avoid these very expensive and very exhaustive clinical trials, although human intervention studies will ultimately be required to confirm the immune effects hypothesized.

Below we describe some of the mechanisms that are currently thought to be at the basis of the immunomodulatory effects of *L. casei* Shirota.

4.1. Natural killer cell activity

Natural killer (NK) cells are part of the innate immune system. They are large lymphocytes lacking antigen-specific receptors, that target and kill abnormal cells including certain tumour cells and cells infected by viruses. NK cells are therefore key players for the immune system to fight viral infections such as the common cold and flu (Brandstadter & Yang 2011), as well as in the prevention of certain types of cancer (Imai *et al.* 2000). Lifestyle and environmental factors, such as obesity and cigarette smoking, are known to reduce NK cell activity. Maintaining or strengthening NK cell activity is therefore considered an important mechanism in support of a functional immune system, and the effect of *L. casei* Shirota on NK cell activity has been explored in healthy adults (Shida *et al.* 2017 [see page 16], Harbige *et al.* 2016, Nagao *et al.* 2000), older adults (Dong *et al.* 2013, Takeda & Okumura 2007) and smokers (Morimoto *et al.* 2005, Reale *et al.* 2012).

Example Study

Effect on natural killer cell activity in male cigarette smoking adults
Reale *et al.* (2012) *Brit J Nutr* **108**(2):308-314

Methods

This double-blind placebo-controlled, randomised study looked at the effect of *L. casei* Shirota on natural killer (NK) cell activity in 72 healthy Italian blue collar male cigarette smoking adults. Subjects were given *L. casei* Shirota powder, 4 sachets/d, for 3 weeks (n=36) (10^{10} viable cells/sachet) or a placebo (n=36) for three weeks. Baseline and post-consumption samples of peripheral blood mononuclear cells were taken in order to measure NK cell activity and CD16+ lymphocytes (the latter indicate induction of cytotoxic activity of NK and other immune cells). Importantly, before intake, NK cytotoxic activity was found to be inversely correlated with the number of cigarettes smoked.

Results

L. casei Shirota intake was associated with a significant increase in NK cytotoxic activity as well as an increase in CD16+ cells (both $P<0.001$), indicating that the probiotic helped to increase NK activity, which was otherwise lower due to smoking.

4. Immune mechanisms (continued)

4.2. Secretorial IgA

Immunoglobulin A (IgA) is an antibody that plays a crucial role in the immune function of mucous membranes, providing protection against toxins and pathogenic microorganisms, and inhibiting inflammatory effects of other immunoglobulins. It also plays an important role in protecting against upper respiratory tract infections (URTI). There is growing evidence to support an immunomodulatory effect of probiotics, specifically through the modulation of IgA levels, although such effects are strain specific (Ashraf & Shah 2014).

Studies conducted with *L. casei* Shirota in healthy adults (Harbige *et al*. 2016, O'Connell *et al*. 2010) and elite athletes (Gleeson *et al*. 2011) have shown that consumption of this probiotic can help to maintain salivary IgA levels, which in the athletes' study was associated with a reduced incidence of URTI as detailed here.

Example Study

Effect on incidence of common colds and salivary IgA in athletes
Gleeson *et al.* (2011) *Int J Sport Nutr Exerc Metab* **21**:55-64.

Methods

This double-blind, placebo-controlled study conducted in the UK aimed to investigate the effects of the probiotic *L. casei* Shirota on the incidence of upper respiratory-tract infections (URTI) and immune markers, in 84 male and female athletes engaged in endurance-based physical activity. Subjects were randomised to consume either a fermented milk drink containing *L. casei* Shirota (6.5×10^9 CFU) (n=42) or placebo (n=42) daily for 16 weeks. Fifty-eight subjects completed the study (probiotic n=32, placebo n=26).

Results

The proportion of subjects who experienced 1 or more weeks with URTI symptoms was 27% lower in the probiotic group compared to placebo (0.66 vs 0.90 respectively, P=0.021) and the mean number of URTI episodes was also significantly lower in the probiotics group compared to the placebo (1.2 vs 2.1 respectively, P<0.01).

The main finding from the immune analysis was that salivary IgA concentrations were significantly higher in the probiotic group than the placebo group at both week 8 (P=0.03) and 16 (P=0.01).

Thus, daily consumption of *L. casei* Shirota appeared to reduce the frequency of URTI in this group of athletes, which is likely to be attributable to the maintenance of salivary IgA levels which would otherwise have decreased during a winter period of intense sports training.

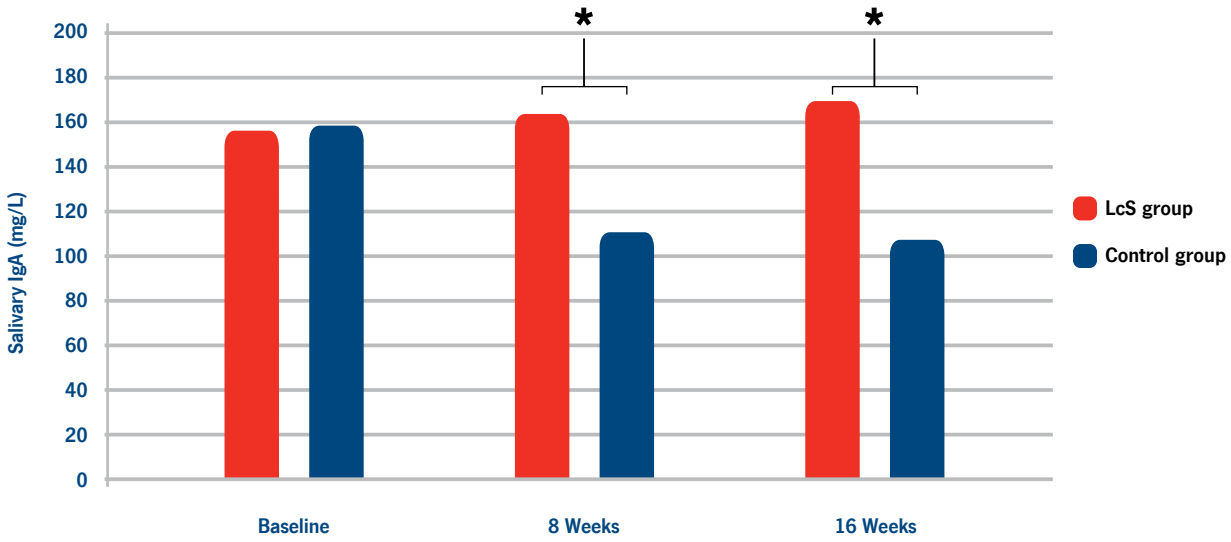


Figure 7 | Effect of *L. casei* Shirota on salivary IgA concentrations

4. Immune mechanisms (continued)

4.3. Dendritic cell, T-cell and cytokine activity

Dendritic cells (DC) and T-cells (or T-lymphocytes) are an essential part of the immune system, involved in identifying foreign substances such as pathogens and initiating a specific immune response. DC are specialised antigen-presenting cells capable of driving antigen-specific T-cell responses, and they determine whether those T-cell responses will be tolerogenic or immunogenic. Cytokines are cell signalling molecules, produced by a broad range of cells including T-cells, that are key in regulating the immune system's response to both infection and inflammation.

In addition to the immunomodulatory effects of probiotics by the modulation of IgA concentrations, some strains of probiotics will also have positive effects on DC, T-cells and cytokines (Ashraf & Shah 2014), including *L. casei* Shirota, as shown further.

In 2011, the hypothesis was raised that *L. casei* Shirota could induce immunoregulatory properties in DC and T-cells, and possibly divert effector T-cells away from intestinal sites to reduce inflammation (Mann *et al.* 2011). This has been further explored in inflammatory bowel disease, specifically ulcerative colitis (UC) which is characterised by a dysregulated intestinal immune response to the gut microbiota (Mitsuyama *et al.* 2008).

Example Study

Conditioning intestinal DCs with *L. casei* Shirota in UC patients
Mann *et al.* (2014) *Inflamm Bowel Dis* **20**:2299-2307.

Method

Intestinal DCs were isolated from healthy controls and from patients with active ulcerative colitis (UC) (biopsies were taken from both inflamed and non-inflamed colon in UC patients), and conditioned with *L. casei* Shirota.

Results

The researchers identified skewed DC subsets in UC patients, specifically the loss of CD103+ lymph-node homing DCs important in generating regulatory T-cells that are anti-inflammatory, and infiltrates of $CD11c^-$ (non-myeloid marker) DCs with enhanced expression of Toll-like receptors for bacterial recognition in UC patients. The authors hypothesized this likely contributes to a reduced T-cell stimulatory capacity in the inflamed tissue of the UC colon.

Conditioning intestinal DCs with the probiotic strain *L. casei* Shirota in UC partially restored their normal function, as indicated by a reduced Toll-like receptor 2/4 expression and by the restoration of their ability to imprint homing molecules on T-cells and to generate IL-22 production by stimulated T-cells. Results suggested that T-cell dysfunction in UC is indeed driven by DCs and that bacterial conditioning of gut DCs can indirectly manipulate T-cell responses with implications for immunomodulatory effects of the commensal microbiota *in vivo*.

Conclusions

The manipulation of DCs allow DC-specific therapy that may be beneficial in inflammatory bowel disease.

Further supportive studies

Downregulation of inflammatory responses by *L. casei* Shirota has been shown in other applications and through other mechanisms. The strain was shown to provoke anti-inflammatory and immunoregulatory effects in Peyer's patches (Chiba *et al.* 2010). In macrophages stimulated by *L. casei* Shirota, the IL-12/IL-10 cytokine ratio can vary with the bacterial signals they encounter in the environment (Shida *et al.* 2009, Shida *et al.* 2011), an effect also observed *in vitro* for e.g. the activity of the pro-/ anti-inflammatory transcription factor NFκB (Habil *et al.* 2012).

More recently, a preliminary study investigating whether the anti-inflammatory properties of *L. casei* Shirota could reduce the inflammatory index of patients with HIV suffering from persisting inflammation, showed positive effects on T-cells and CD56+ cells. Furthermore, cytokine markers on the serum and mRNA level changed significantly, and inflammation and cardiovascular risk decreased. The researchers concluded that *L. casei* Shirota may modulate immunological parameters and may therefore be an inexpensive and practical strategy to support the immune function of patients with HIV (Falasca *et al.* 2015).

The effects of *L. casei* Shirota on immune profiles and intestinal microbial translocation in children with HIV has also been investigated and the findings suggest that short-term *L. casei* Shirota consumption is a safe supportive approach with immunological and virological benefits in children with HIV (Ishizaki *et al.* 2017).

4. Immune mechanisms (continued)

4.4 Serum CRP levels

It is generally accepted that the C-Reactive Protein (CRP) in the blood is increased under conditions of inflammation, infection or following a trauma such as surgery or a heart attack. Under normal conditions serum CRP levels are low, requiring a high-sensitivity CRP (hs-CRP) test to identify low levels of inflammation.

Although prior to the 1990's osteoarthritis (OA) was considered a disease of "wear and tear", it is now well documented that the condition is associated with the presence of inflammatory markers such as CRP. As treatment options are limited, exploring alternative therapies to improve symptoms of OA are sought.

Example Study

Effects on CRP concentrations in osteoarthritis (OA)
Lei *et al*. (2017) *Benef Microbes* **8(5)**:697-703.

Methods

This study investigated the effect of *L. casei* Shirota on 537 patients with knee OA. In this double-blind, placebo-controlled trial, the primary outcome was defined as changes in WOMAC (Western Ontario and McMaster Universities Osteoarthritis Index) symptoms and VAS (visual analogue scale) pain scores, and hs-CRP was measured as a secondary outcome.

Results

After 6 months of treatment, WOMAC and VAS scores, as well as hs-CRP levels were significantly improved in the *L. casei* Shirota group of patients compared to the placebo group. A strong linear correlation was observed between serum hs-CRP levels and WOMAC and VAS scores.

Conclusions

It was concluded that consumption of a probiotic may be a therapeutic option in the clinical management of knee OA, which is traditionally challenging due to inefficacy and adverse effects of the current therapies.

4.5 Further research on mechanisms of action

Mechanisms underlying the immune effects of *L. casei* Shirota tend to be determined by different molecules. The cell wall of *L. casei* Shirota is responsible for the induction of the anti-infection cytokine IL-12, able to stimulate Th1 cell development and activate NK cells (Shida *et al.* 2011). Interestingly, this IL-12 production by macrophages is modified when other bacteria or their cell components are also present (Kaji *et al*. 2010).

A study conducted in the UK showed that different immune mechanisms can be active simultaneously. In the study by Harbige *et al.* (2016) the immunological effects of a fermented milk drink containing *L. casei* Shirota was investigated in healthy adult volunteers, using an intermittent regime with a 4-week ingestion period, 6 weeks with no probiotic, followed by a further secondary period of 4 weeks with the probiotic. Using this regime an increased expression of the CD69 activation marker on circulating T-cells and NK cells was found, as well as a higher mucosal salivary IFN-γ, IgA1 and IgA2 concentration, with evidence of secondary boosting at week 14. There was also a decrease in IL-4 in $CD3^{+}\beta7^{+}$ integrin cells and a CD14+ cell anti-inflammatory cytokine profile was induced. Interestingly, there was no effect on systemic circulating influenza A specific IgA or IgG, and tetanus specific IgG antibody levels with the schedule and dose used.

5. Cancer

Many different biomarkers indicative of different mechanisms of activity have been used in probiotic studies relating to cancer. The research for *L. casei* Shirota ranges from genotoxicity tests to retrospective epidemiological studies, as well as human intervention studies, showing:

- Reduction of harmful substances (carcinogenic compounds and enzymes) in the gut (De Preter *et al.* 2004, De Preter *et al.* 2007, De Preter *et al.* 2008, De Preter *et al.* 2011).
- Restoration of NK cell activity (Morimoto *et al.* 2005, Reale *et al.* 2012) (see section 4).
- Reduction of human papillomavirus infection rate (Verhoeven *et al*. 2012) (see section 3).
- Positive clinical outcomes predominantly in colorectal cancer (Ishikawa *et al*. 2005) and bladder cancer (Aso *et al.* 1992, Aso *et al*. 1995), but there are also positive outcomes relating to cervical cancer (Verhoeven *et al.* 2012), breast cancer (Toi *et al.* 2013), biliary and liver cancer (reduction of post-operative infections) (Eguchi *et al.* 2010, Kanazawa *et al*. 2005, Sugawara *et al.* 2006), lung (Masuno *et al.* 1989, Masuno *et al.* 1991, Masuno *et al.* 1994), and uterine cancer (Okawa *et al*. 1989, Okawa *et al.* 1993).

The effect of a synbiotic, containing *L. casei* Shirota alongside a prebiotic, has also been investigated in oesophageal patients receiving chemotherapy (Motoori *et al.* 2017)

5.1. Colorectal cancer

Worldwide colorectal cancer (CRC) is the second most common cancer in women and third in men (Ferlay *et al.* 2015). Whilst several aspects of lifestyle play a role in this cancer's aetiology, it is now widely believed that components of the intestinal microbiota may be involved in CRC carcinogenesis. The following long-term clinical trial is a highlight of the broad range of cancer studies with *L. casei* Shirota.

Example Study

Effect on CRC tumours (four-year trial)
Ishikawa *et al.* (2005) *Int J Cancer* **116**:762-767.

Method

In this randomised trial, 398 subjects who had previously had at least two colorectal tumours removed, were assigned for four years to one of the following dietary interventions: (i) dietary advice with consumption of wheat bran (7.5 g three times a day); (ii) *L. casei* Shirota (10^{10} viable cells/g three times a day as powder); (iii) both; or (iv) neither. Consultations occurred every three months. The primary endpoint was presence or absence of new tumours after two and four years' intervention, detected by colonoscopy.

Results

After two years, multivariate adjusted OR (95% CI) for tumour occurrence was 0.76 (0.50-1.15) for the *L. casei* Shirota group but 1.31 (0.87-1.98) in the wheat bran group. After four years, in the *L. casei* Shirota group a significant decrease was observed for occurrence of colorectal tumours with moderate to severe atypia (OR 0.65, 95% CI 0.43-0.98). Wheat bran consumption resulted in a significantly higher incidence of tumours > 3mm. Groups consuming wheat bran plus *L. casei* Shirota had lower tumour occurrence compared to those without any intervention, but higher compared to those on single dietary intervention.

Conclusions

The authors concluded that intake of *L. casei* Shirota appeared to suppress development of colorectal tumours. There was a statistically significant association between probiotic consumption and reduced risk of development of tumours with moderate to severe atypia.

5.2. Bladder cancer

L. casei Shirota research relating to bladder cancer includes epidemiological and human intervention studies. For example a retrospective case-control study by Ohashi *et al.* (2002) compared 180 bladder cancer cases with 445 population matched controls in relation to ingestion of milk fermented with *L. casei* Shirota over the past 10-15 years. A negative correlation was found, indicating habitual intake of the lactic acid bacteria was associated with lower risk of bladder cancer.

5. Cancer (continued)

Example Study

Effect on recurrence of superficial bladder cancer
Aso *et al*. (1995) *Eur Urol* **27**:104-109.

Method

This randomised, double-blind, placebo-controlled trial involved 138 patients who had undergone previous transurethral resection of bladder cancer. Prior to randomisation, they were stratified according to those with primary multiple tumours, those with recurrent single tumour, and those with recurrent multiple tumours. For one year or until tumour recurrence, subjects consumed either (i) 1 g of powder containing 1 x 10^{10} CFU of *L. casei* Shirota three times daily or (ii) a placebo.

During the study period, no other anti-cancer therapy was given. Tumour recurrence was monitored by quarterly cytological analysis of urine and endoscopic analysis. Routine haematology, biochemistry and urine tests were conducted before surgery at enrolment, at one and three months after enrolment, and thereafter every three months.

Results

Compared to placebo, *L. casei* Shirota was more effective in reducing risk of tumour development in patients with primary multiple tumours and those with recurrent single tumours but not in patients with recurrent multiple tumours. When data from patients with primary multiple tumours and with recurrent single tumours were combined for analysis, this showed that the 50% recurrence-free period was longer in the probiotic group (688 days) compared to the control (543 days), which was equivalent to a 1.3-fold increase in time (P=0.08). Multivariate life-table analysis of this combined group also showed a significantly better outcome for those on probiotic (P=0.01).

Conclusions

The authors concluded that the *L. casei* Shirota preparation was safe and might help reduce the risk of recurrence of superficial bladder cancer.

5.3. Cervical cancer risk: Human papillomavirus infection

Globally, cervical cancer is the fourth most common cancer in women (Ferlay *et al.* 2015). Cervical cancer is associated with infection by a member of the family of human papillomaviruses (HPV): HPV-DNA can be found in 99.7% of all cervical cancers. Low-grade squamous intraepithelial lesions are closely associated with infection with certain high-risk HPV; the clearance of both shows a close temporal relationship (Syrjänen 2007).

Example Study

Effect on HPV-associated pre-cancerous abnormalities (adults)
Verhoeven *et al.* (2013) *Eur J Cancer Prev* **22**(1):46-51.

Method

This open-label, controlled study in Belgium investigated whether *L. casei* Shirota could influence clearance of HPV infection and associated pre-cancerous abnormalities (low-grade squamous intraepithelial lesions; LSIL). For six months, the researchers followed 54 women diagnosed with HPV+ LSIL in their PAP smear, who had been allocated to take either a daily fermented milk drink with *L. casei* Shirota or no intervention. Efficacy was assessed by comparing PAP smear and HPV status, at baseline and after six months.

Results

The women in the *L. casei* Shirota group had twice as high a chance of cytological abnormalities becoming cleared (P=0.05) compared to the control group. HPV was cleared in 29% of the *L. casei* Shirota group compared to 19% in the control group (P=0.41) (see Figure 8).

Conclusions

The authors concluded that this indicated that *L. casei* Shirota promotes clearance of HPV-related cytological abnormalities, a potentially new way of managing cervical cancer precursors.

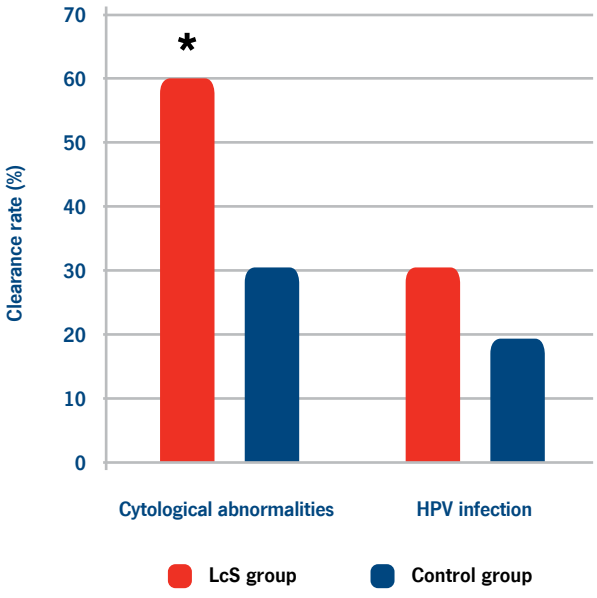


Figure 8 | Effect of *L. casei* Shirota consumption on clearance rate of cytological abnormalities and HPV infection.

5. Cancer (continued)

5.4. Breast cancer

Breast cancer (which can also affect men) is the most common cancer worldwide (Ferlay *et al.* 2015). In 2012, there were 464,000 cases of breast cancer in women across Europe (Ferlay *et al.* 2013) and therefore establishing cancer control actions for breast cancer in Europe is a priority.

Example Study

Breast cancer incidence: effects of soy isoflavone and *L. casei* Shirota

Toi *et al.* (2013) *Curr Nutr Food Sci* **9**(4)**:**194-200.

Method

This population-based case-controlled study aimed to evaluate how consumption of beverages containing *L. casei* Shirota and/or soy isoflavone since adolescence affected the risk of later developing breast cancer. The researchers matched 306 women with breast cancer and 662 controls aged 40-55 years. Diet, lifestyle and other breast cancer risk factors were analysed by means of self-administered questionnaire and interview.

Results

The odds ratio of regular *L. casei* Shirota consumption (more than 4 times per week) for breast cancer was 0.65 (P=0.048). Furthermore, the greater the consumption of soy, the lower the odds ratio of breast cancer became.

Conclusions

The authors concluded that regular consumption of *L. casei* Shirota and soy isoflavones since adolescence was inversely associated with the incidence of breast cancer in Japanese women.

6. Emerging areas of research

Research into *L. casei* Shirota has been conducted for over 85 years, and by researchers all over the world so it is not surprising that a broad range of effects has been investigated. A few studies are described here; for further examples try the suggested searches on page 28.

6.1 Liver disease

Liver disease is a significant burden in Europe, and associated mortality is at least comparable with other diseases that are considered to be of major public health concern (Blachier *et al*. 2013). Patients with liver disease generally have increased susceptibility to infection, and once infected have increased in-hospital mortality. The importance of the intestinal microbiota in supporting the immune response and defence against infection has led to probiotic research in this area. A range of research has examined the effects of *L. casei* Shirota (in some cases as a synbiotic) for patients with liver disease, including gut microbiota and liver function (Koga *et al.* 2012), infectious complications (Eguchi *et al.* 2010, Kanazawa *et al.* 2005, Sugawara *et al.* 2006, Usami *et al.* 2011), and immune function (Stadlbauer *et al.* 2008). There have also been case reports (Shimizu *et al.* 2012).

The two primary lines of research with *L. casei* Shirota are (i) studies with a synbiotic combination to prevent infectious complications as a result of surgery and (ii) restoration of immune function.

6.1.1. Infectious complications post-surgery

Example Study

Effect of perioperative synbiotic treatment on infectious complications after liver transplantation
Eguchi *et al*. (2010) *Am J Surgery* **201**(4):498-502.

Method

This was a prospective randomised study in 50 patients undergoing living donor liver transplantation (LDLT) – LDLT patients suffer higher portal hypertension post-surgery compared with whole-liver transplantation. Patients were randomised to a synbiotic or no intervention. The synbiotic was a combination of probiotic strains (*Bifidobacterium breve* Yakult, *L. casei* Shirota) and prebiotic galactooligosaccharide, taken for two days before the operation and two weeks after.

As standard procedure, patients received intravenous prophylaxis for four days (amoxicillin and cefotiam). Immunosuppressive treatments were also given, either dual or triple, including tacrolimus or cyclosporine A, prednisolone, and/or mycophenolate mofetil. Twenty-four hours after the operation, patients received enteral nutrition through a jejunostomy.

Results

An episode of infectious complication occurred in one patient in the synbiotic group (4%) compared to six cases in the control group (24%: three cases of sepsis, three urinary tract infections with *Enterococcus*) (P=0.033).

6.1.2. Restoration of immune function

Alcoholic hepatitis is considered a pro-inflammatory condition, treated with steroids and anti-TNF-α, yet deaths are mainly due to sepsis, indicating a failure of the immune response against infection. Neutrophils are primed with an increase in activated oxidative burst yet have impaired function. This defective immune response is associated with significantly higher risk of infection, organ failure and mortality. Researchers at University College London investigated the theory that this was due to impaired gut permeability leading to endotoxemia. They reasoned that beneficial modulation of the gut microbiota with a probiotic might reverse this situation and thus restore neutrophil function.

Example Study

Effect on poor immune response of patients with compensated alcoholic cirrhosis
Stadlbauer *et al.* (2008) *J Hepatol* **48**:945-951.

Method

In this proof of concept, open-label study, 12 patients consumed *L. casei* Shirota (6.5 x 10^9 CFU) three times daily in the form of a fermented milk drink for four weeks. Immune parameters were measured at baseline and after four weeks. Data were compared with 13 healthy controls and 8 similar patients who did not consume probiotics.

Results

At baseline, neutrophil phagocytic capacity of patients was lower than the healthy controls (73% vs. 98%; P<0.05). Plasma levels of soluble TNF-receptor (sTNFR)-1 and -2, and IL-10 were significantly higher in patients compared to healthy controls; this did not change during the study. TLR2, TLR4 and TLR9 were also overexpressed in the patients.

L. casei Shirota effects on patients compared to the control patients were as follows: (i) neutrophil phagocytic capacity and TLR4 expression normalised (P<0.05); (ii) levels of sTNFR -1 and -2, and IL-10 (*ex vivo* and endotoxin-stimulated) were lower in the probiotic group (P<0.05).

Conclusions

The authors concluded these data provide proof of the concept that probiotics can restore neutrophil phagocytic capacity in cirrhosis.

6. Emerging areas of research (continued)

6.2 Metabolic disease

Metabolic disease (or metabolic syndrome) is the term given to a group of risk factors that increase risk of heart disease, stroke and diabetes. Such factors include abdominal adiposity, dyslipidaemia, hypertension and hyperglycaemia. These metabolic risk factors are increasingly linked to dysbiosis of the intestinal microbiota.

High fat, high sugar diets have increasingly been shown to alter microbiota compositions substantially in animals, and several researchers have now shown that the transplant of obesogenic dysbiotic microbes into germ-free mice results in an increase in fat mass (Turnbaugh *et al.* 2006, Turnbaugh *et al*. 2009).

Such metabolic diseases and risk factors result in the activation of pro-inflammatory mechanisms and metabolic endotoxemia, which exacerbates the problem through promoting insulin resistance and other metabolic abnormalities. As probiotics have been described to reduce inflammation and intestinal permeability, and therefore have the potential to decrease metabolic endotoxemia, the application of probiotics as biotherapeutics are therefore considered as valuable alternatives to medical therapeutic approaches.

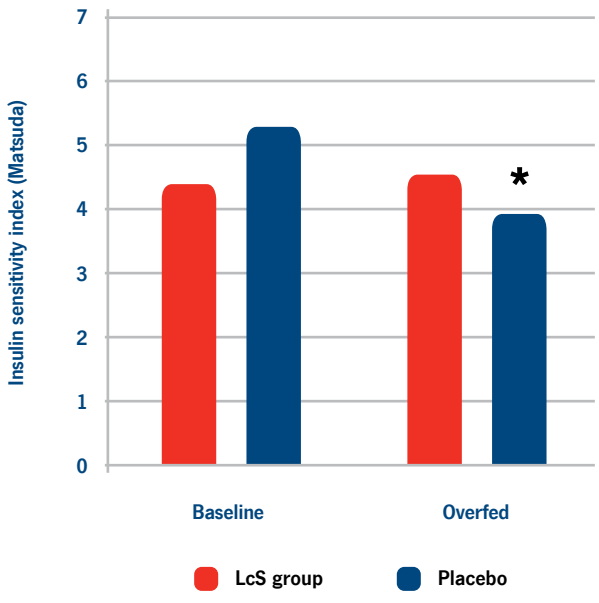
Although use of probiotics remains an emerging area of research, there have been some interesting findings to report. Early research with extracts of *L. casei* Shirota suggested a benefit in reducing hypertension (Nakajima *et al.* 1995, Watanuki *et al.* 1999) and provided a potential mechanism (Gonzalez-Gonzalez *et al*. 2011). A recent observational study concluded that the risk of developing hypertension is substantially lower in those who consume *L. casei* Shirota at least 3 times a week (Aoyagi *et al.* 2016). Metabolic effects of *L. casei* Shirota have also been studied in obese children (Nagata *et al.* 2017), obese pre-diabetic men (Naito *et al*. 2018) and patients with type 2 diabetes (Sato *et al.* 2017). Furthermore, a pilot study conducted in the UK has shown beneficial effects on blood glucose and insulin resistance in a healthy student population, as detailed here.

Example Study

Maintenance of blood glucose and insulin sensitivity
Hulston *et al.* (2015) *Br J Nutr* **113**: 596-602.

Method

This randomised, open-label, controlled study conducted at Loughborough University in the UK, investigated whether *L. casei* Shirota can reduce the occurrence of insulin resistance induced by a short-term overfeeding. Seventeen healthy, normal weight and physically active participants were randomly assigned to a group receiving *L. casei* Shirota (1.3×10^{10} CFU/day x 4 weeks) or to a control group with no supplementation. Both groups ate their usual diet for 3 weeks and were challenged with a high-fat (65% of energy), high-energy (50% increase in energy intake) diet for one week. An oral glucose tolerance test (OGTT) was performed before and after the overfeeding. Blood glucose and insulin were also measured at the same timepoints.



Results

The high-fat, high-energy diet led to an increase in weight ($P<0.05$), fasting plasma glucose ($P<0.05$) and glucose area under the curve AUC in the OGTT by 10% ($P<0.05$), and a 27% decrease in insulin sensitivity ($P<0.05$) in the control group. Whereas the increase in weight was not significant, and fasting plasma glucose, glucose AUC and insulin sensitivity did not change in the *L. casei* Shirota group.

Conclusions

The consumption of *L. casei* Shirota before and during a high-fat, high-energy diet may be useful in ameliorating diet-induced insulin resistance and potentially also subsequent diet-induced metabolic diseases. This study is being followed up by a larger cohort to confirm the findings.

Figure 9 | Effect of *L. casei* Shirota on insulin sensitivity

6. Emerging areas of research (continued)

6.3 Gut-brain: mood & stress response

Headlines such as "Probiotics and Your Memory: The Gut-Brain Connection", "There's a Brain in Your Gut" and "The Gut-Brain Axis: The Missing Link in Depression" have been highlighting new insights in the importance of the gut – brain connection. The gut-brain axis refers to a bidirectional communication between the central and the enteric nervous system. It has the potential to influence emotional and cognitive centres in the brain directly from the intestinal neurological environment. Besides neural influence also endocrine, immune and humoral moderators are being identified (e.g. serotonin or gamma-aminobutyric acid). Possible applications of these new insights may include autism, anxiety and depressive disorders, as well as Parkinson and Alzheimer diseases (Dinan & Cryan 2017a, Dinan & Cryan 2017b).

While a lot of the initial research on the use of probiotics to improve mood and/or stress was done on animals, there are more and more studies that show an effect in humans and the term "psychobiotics" has even been coined, referring to possible probiotics that could have an effect on mood and depression (Dinan *et al.* 2013).

Studies conducted using *L. casei* Shirota have thus far focused on mood in individuals with chronic fatigue syndrome (Rao *et al.* 2009) and healthy adults (detailed below). There have also been double-blind placebo-controlled trials conducted in Japan than have shown positive effects of consuming *L. casei* Shirota for eight weeks prior to an academic exam on stress-induced abdominal dysfunction, stress markers (psychological and physiological), sleep quality and the composition of the gut microbiota (Kato-Kataoka *et al.* 2016a, Kato-Kataoka *et al.* 2016b, Takada *et al.* 2017).

Example Study

Effect on mood and cognition (in healthy adults)
Benton *et al.* (2007) *Eur J Clin Nutr* **61**:355-361

Method

This double-blind placebo-controlled study conducted in the UK involved 132 healthy adults (mean age: 61.8 yrs) living in the community. Every day for three weeks, the volunteers drank one fermented milk drink containing *L. casei* Shirota (minimum 6.5 x 10^9 CFU) or a milk-based placebo of similar colour and taste. Mood and cognition was assessed throughout the drinking period.

Results

After 20 days, the mood in those who were more depressed at baseline (the bottom third of the participants based on the profile of mood states) had significantly improved (P<0.04).

Example Study

Effect on markers of stress and stress-induced symptoms in students taking exams
Kato-Kataoka *et al.* 2016 *Appl Environ Microbiol* **82**(12):3649-3658.

Method

This double-blind, placebo-controlled study investigated the effect of *L. casei* Shirota on psychological and physiological stress responses of healthy medical students ahead of an exam. The participants consumed either a fermented milk drink containing *L. casei* Shirota (n=23; 10^{11} CFU/day) or a placebo (n=24) daily for 8 weeks up until the day before their exam. The primary focus was on stress-induced abdominal dysfunction and stress markers (psychological and physiological) measured at 8 weeks, 2 weeks and 1 day before the exam, and immediately after and 2 weeks after the exam. They also examined whether *L. casei* Shirota administration affected stress-induced changes in the composition of the gut microbiota and stress-responsive gene expression in peripheral leukocytes.

Results

The scores on stress and abdominal dysfunction were significantly lower in the *L. casei* Shirota group than the placebo group throughout the intervention (p<0.05 for both parameters). *L. casei* Shirota not only prevented the onset of symptoms, but improved abdominal discomfort and pain in those who suffered from them before the intervention. The *L. casei* Shirota group also exhibited reduced number of stress-responsive genes in white blood cells one day before (P<0.001) and immediately after the examination (P<0.05) compared to placebo and preserved the diversity of the microbiota in the gut. Salivary cortisol (a marker of stress) increased significantly in the placebo group on the day before examination compared with baseline (P<0.05), but not in the *L. casei* Shirota group.

Conclusion

Daily *L. casei* Shirota administration may attenuate the biological response to stress when confronted with academic stress such as an examination, by preserving a diverse microbiota which may be associated with a reduction in feelings of stress and abdominal dysfunction.

6. Emerging areas of research (continued)

6.4 Miscellaneous areas of research

There is a surprising and exciting breadth of new emerging areas of research conducted with *L. casei* Shirota. Studies include those conducted by researchers in the company's research institutes in Japan and Belgium, as well as collaborative research with hospitals, universities and institutes worldwide. Studies are also done by completely independent groups. Here are some further areas of emerging research:

Oral health: The effects of *L. casei* Shirota consumption have been investigated in several human studies, including those investigating the oral health of people with dentures (Sutula *et al.* 2012) and their own teeth (Sutula *et al.* 2013); the presence of *Candida* in the oral cavity of elderly people (Mendonça *et al*. 2013); the presence of *Streptococcus mutans* and cavities in children (Lin *et al.* 2014, Lin *et al* 2017) and gingivitis (Slawik *et al.* 2011).

Lactose intolerance: A study in 27 patients with lactose maldigestion and intolerance, found that a four-week intervention with a mixture of *L. casei* Shirota and *B. breve* Yakult reduced symptom scores and breath hydrogen after lactose ingestion (Almeida *et al*. 2012).

Osteoarthritis: A double-blind, placebo controlled trial in 537 patients with knee osteoarthritis found that after 6 weeks of *L. casei* Shirota there was a significant improvement in quality of life (WOMAC) and pain (VAS) scores, as well as hs-CRP levels, compared to the placebo group (Lei *et al.* 2017, see page 21).

Bone density: In a double-blind, placebo-controlled trial, 417 elderly patients with acute distal radius fractures were randomised to receive either *L. casei* Shirota or a placebo for 6 months. Throughout the intervention, several markers improved at a significantly faster rate in the probiotic group compared to placebo, including pain scores, range of motion and grip strength (Lei *et al*. 2016).

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