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Health effects of probiotics and prebiotics
A literature review on human studies

By Henrik Andersson, Nils-Georg Asp, Åke Bruce, Stefan Roos, Torkel Wadström and Agnes E. Wold

ABSTRACT

Human studies on health effects of probiotics and prebiotics were reviewed and evaluated. The main results can be summaries as follows: Certain probiotic lactobacilli may improve lactose digestion and reduce symptoms of lactose intolerance. The effect of probiotics on serum cholesterol is still inconclusive. Animal studies showing triacylglycerol-lowering effects of prebiotics need confirmation in humans. Data on effects of probiotics on constipation are not convincing, whereas inulin has dose-related laxating effect. Effects of a probiotic drink have been reported on symptoms in irritable bowel syndrome, but more studies are needed for firm conclusions. A significant shortening of acute watery rotavirus-included diarrhoea has been demonstrated for two lactobacilli, whereas possible effects on the risk of getting traveller's diarrhoea need further studies. There are promising indications that probiotics could be useful against antibiotic-associated diarrhoea, and a yeast preparation has been shown to reduce the risk of relapsing *Clostridium difficile* diarrhoea. Promising results from studies on the effect of probiotic products in the treatment of gastritis and inflammatory bowel disease should encourage further studies with pro-, pre- and synbiotic foods. Certain prebiotic oligosaccharides may increase calcium absorption. Probiotics can be regarded as safe although occasional infections have been reported in immunosuppressed patients. Prebiotics such as fructans may cause dose dependent gastrointestinal side-effects.

The documentation of health-promoting effects of probiotic and prebiotic products is rapidly increasing. The food industry that develops pro- and prebiotic products should increase their efforts to develop high quality research and well-designed clinical trials on ordinary food products. This area is of great importance for improving human health.

Introduction

There is increasing evidence that the composition and metabolic effects of the gastrointestinal microflora are of key importance for human health. In addition to promoting normal gastrointestinal functions and protecting from infections, the microflora also seems to exert important effects on systemic metabolism and immune functions.

The definitions of *probiotics* as "live microbial food ingredients that are beneficial to health", *prebiotics* as "non-digestible food components that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, that have the potential to improve host health", and *synbiotics* as "mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract" are generally accepted (1).

Microorganisms representing many different genera have been used as probiotics. Most of the efforts have been focused on lactobacilli and bifidobacteria, but also enterococci and the yeast *Saccharomyces boulardii* have received attention. The term "prebiotics" was coined at a time when the first studies appeared showing that fructo-oligosaccharides – undigestible in the small intestine – could be utilised only by a few bacterial species, notably bifidobacteria, and that feeding such oligosaccharides to experimental animals and man increased the count of bifidobacteria in the intestinal content (2). Much research on oligosaccharides, notably fructo-oligosaccharides and inulin, has been performed recently, including European Commission sponsored projects (3). It should be noted, however, that the stimulation of certain microorganisms by different carbohydrates *in vivo* does not appear as selective as indicated by *in vitro* studies Thus, resistant starch and non-starch polysaccharides have also shown a capacity to stimulate bifidobacteria *in vivo* (4). A question which must be addressed, however, is whether bifidobacteria in thousands really improve host health.

Searching the literature for human effect studies

An *ad hoc* committee of Swedish scientists, the authors of this article, was formed in the spring 2000 jointly by the Expert Group on Diet and Health of the National Food Administration and the Research Board of SNF Swedish Nutrition Foundation. The task was to review and evaluate human studies on the health effects of probiotics and prebiotics.

The objective was to find all original articles in Medline published until autumn 2000 on human effect studies with pro- and prebiotics. The words used in the searches were different combinations of the following terms: *Lactobacillus*, *Bifidobacterium*, probiotics, different probiotic bacteria or brand names (*Lactobacillus* GG, *Lactobacillus reuteri, Lactobacillus plantarum* 299v, *Shirota, Lactobacillus acidophilus, LA1, NCFB 1748, Lactococcus lactis L1A, Bb12, Gaio, Lactobacillus rham-*

Henrik Andersson, Prof., Dept of Clinical Nutrition, Göteborg University, **Nils-Georg Asp**, Prof., Applied Nutrition and Food Chemistry, Lund University, **Åke Bruce**, Prof., National Food Administration, Uppsala, **Stefan Roos**, Dr, Dept of Microbiology, Swedish University of Agricultural Sciences, Uppsala, **Torkel Wadström**, Prof., Dept of Medical Microbiology and Infectious Diseases, Lund University, **Agnes Wold,** Assoc Prof., Dept of Clinical Immunology, Göteborg University, Sweden.

Correspondence: Nils-Georg Asp, SNF Swedish Nutrition Foundation, Ideon, SE-223 70 Lund, Sweden. E-mail: asp@snf.ideon.se

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nosus), prebiotics, inulin, oligofructose, diarrhoea, lactose, blood lipids, cholesterol, irritable bowel syndrome (IBS), cancer, mucositis, inflammatory bowel disease (IBD), Chrohn's disease, inflammation, allergy, safety.

To find additional articles, and also as a control of our search, reference lists of several recent review articles about pro- and prebiotics were used. Furthermore, companies with pro- or prebiotic foods on the Swedish market were asked to send documentation already published or in press. The articles were sorted after the studied effect into the following groups:

• Lactose intolerance • Blood lipids • Hypertension • Constipation
• Irritable Bowel Syndrome (IBS) • Diarrhoea • Gastritis and reflux disease
• Inflammatory Bowel Disease (IBD) • Cancer prevention
• Prebiotics and mineral absorption • Safety

The papers were critically reviewed by the members of the group. The focus of the examination has been studies using probiotic or prebiotic food products, but also studies with lyophilised bacteria have been considered when relevant.

Some studies are listed in Tables 1-4 on pages 66-75. Those references are marked (*) in the following text and in the reference list. Articles are evaluated in the Tables (column 13) as + low quality; ++ medium quality; +++ high quality.

Due to the complexity of effects on immune functions, a more in-depth review on this topic is presented separately (5).

Lactose intolerance

Lactose is the predominant carbohydrate in milk. The presence of sufficient lactase activity in the small bowel mucosa is necessary for the newborn child to absorb lactose from breast milk. This enzyme is, however, dramatically reduced in adult life in the majority of people. The genes that enable the activity of lactase to remain high prevail mainly in the white Western populations. In Sweden, e.g., low lactase activity is relatively uncommon. It should be noted, however, that lactose malabsorption often occurs without symptoms of lactose intolerance.

Table 1 (on pages 66,68,69) shows studies on lactose intolerance. Several studies have demonstrated that subjects with low intestinal lactase activity absorb lactose from yoghurt (6*,7*) or milk containing *L. acidophilus* (8*) better than from milk. In another study (9*), however, a four-fold difference in lactase activity between the products had no effect on the digestion and tolerance of lactose. Yoghurt is also better tolerated (gives fewer symptoms) than milk (7*,10*). In these studies breath hydrogen concentrations have been measured, as an indication of bacterial fermentation of undigested lactose. The method is only semi-quantitative and there is considerable variation in how much hydrogen is absorbed and how much passes with the stools.

Mechanisms: Up to about half of the lactose content can be fermented after 11 days storage (11*), and microbial lactase activity enters the small bowel with the fermented product. Subjects with lactose intolerance experience more symptoms also after ingesting fructo-oligosaccharides (12*), indicating increased sensivity to malabsorbed carbohydrates.

Conclusion: Despite the above-mentioned concerns, it seems logical to assume that fermented milk products with specific probiotic lactic acid bacteria improve lactose digestibility and absorption, and may reduce symptoms of lactose intolerance. This conclusion is in agreement with a recent review by Hove et al. (13).

Blood lipids

Probiotics

A number of studies have examined the potential of probiotic products to reduce serum cholesterol levels, some are listed in Table 1 (on pages 66-70). Studies without suitable control or placebo groups, lack of run-in periods or administering very large doses of fermented dairy products (700–5000 ml/day) were excluded. An important consideration in the evaluation of the studies is the fact that participation for a single week in a nutritional trial in itself may result in a reduction of serum cholesterol. Furthermore, the analytical precision of serum cholesterol determination has to be considered.

About half a litre of a yoghurt with *L. acidophilus* (14*,15*) or tablets with viable *L. acidophilus* and *L. bulgaricus* (16*) did not reduce serum cholesterol according to one randomised controlled trial (RCT) (39 subjects) and two randomised crossover studies (219 subjects), but a small effect was seen in two other studies (17*,18*). In a further study, yoghurt did not reduce serum lipids (19).

A product containing *L. plantarum* 299v (ProViva®), tested in a parallel study with 30 subjects (20*) gave a significant reduction in serum cholesterol. However, the test group and the control group had similar cholesterol levels at the end of the study.

A fermented milk product containing *Enterococcus faecium* and *Streptococcus termophilus* (GAIO®) has been tested in three RCT studies with parallel designs (21*-23*) in altogether 214 subjects. The randomisation was not always optimal. A small but significant reduction was found at some time-periods, but no difference was found between the groups after six months in one of the studies (23*). GAIO® has also been tested in a randomised crossover study (24*). A reduction in serum cholesterol was found after six weeks.

Mechanisms: The mechanisms whereby probiotics may reduce serum cholesterol are largely unknown. Certain strains of bacteria, however, have the capacity to assimilate cholesterol *in vitro*. An ileostomy study has shown a reduced absorption of cholesterol from the small bowel after treatment with probiotics (25*).

Conclusion: The effect of probiotics on serum cholesterol is still inconclusive. More long-term studies are required to document a sustained effect. These conclusions are supported by recent reviews (26-27).

Prebiotics

Dietary carbohydrates represent a complex group of food components. Dietary oligosaccharides, strictly defined, are composed of two to nine monomers linked together, but inulin is often included in spite of a chain length larger than this. Non-digestible oligosaccharides may act as substrates for the colonic microflora.

Convincing serum lipid-lowering effects of inulin have been demonstrated in animals; attempts to reproduce similar effects in humans, however, have given conflicting results. One reason may be that animals were given much larger doses of inulin than tolerated in humans. Animal studies have identified inhibition of fatty acid synthesis as the major site of action for the triacylglycerid-lowering effect of inulin. This pathway is, however, relatively inactive in humans (28).

Two RCT studies (29*,30*), including altogether 30 subjects, have shown reductions in serum cholesterol and triglycerides with inulin. Moreover, in one small crossover RCT (31*) on 12 subjects, serum cholesterol and triglycerides were reduced after inulin ingestion. In another crossover RCT in 21 subjects (32*), a reduction of serum cholesterol was found only relative to the control group. In three crossover RCTs (33*-35*) including 96 subjects, no effect in serum cholesterol and triglycerides was found. In another study there was also no effect on plasma lipids, but a decreased basal hepatic glucose production was found with fructo-oligosaccharides (36*). In a recent double-blind RCT parallel design study and 54 subjects there was a trend for the

triacylglycerol level to be lower after 8 weeks on 10 g of inulin (37*). Ileostomy studies have not shown any increase in cholesterol or bile salt excretion from the small bowel by prebiotic treatment (38*), i.e. addition of 17 g of inulin or oligofructose to the diet.

Conclusion: No convincing serum cholesterol-lowering effect can be ascribed to inulin or oligo-fructose from the present studies. The effect on the triacylglycerol level in man remains to be elucidated further.

Hypertension

Two Japanese studies have been published on the effect of fermented milk on blood pressure (Table 1, on pages 67,68,70). In the first study, *Lactobacillus helveticus* and *Saccaharomyces cerevisiae* were used (39*), in the second one *L. casei* TMC 0409 and *Streptococcus thermophilus* TMC 1543 were investigated (40*). In both studies significant reductions in systolic blood pressure were found, and in one study (39*), effects on diastolic blood pressure were also noted. It was suggested that the effect could be due to the formation of certain tripeptides that are inhibitors of ACE (Angio-tension-converting enzyme).

Further studies are needed to test the effect of these peptides and to establish the conditions for their formation.

Constipation

Probiotics

Constipation means complaints with bowel evacuations, low bowel emptying frequency and a slow transit through the large bowel. Based on early reports, different fermented milk products have been claimed to alleviate constipation. Such self-reported information, however, is difficult to evaluate.

Two hundred ml of a *L. rhamnosus* GG fermented whey drink (41*) did not change bowel movement frequency or hardness of stools in a small placebo-controlled study. Similar findings were made with a *L. rhamnosus* GG yoghurt (42). In another study the effect of milk fermented by *L. acidophilus* on constipation in elderly subjects was difficult to evaluate (43*).

Conclusion: The available data on effects on constipation of probiotics are not convincing. Further studies are needed to substantiate such an effect.

Prebiotics

Dietary fibre increases the faecal bulk by two mechanisms: incompletely fermented types of fibre bind water throughout the gastrointestinal tract, whereas readily fermented types of fibre contribute by increasing the microbial mass (44). Since oligosacharides are completely fermented, their bulking effect would be expected to occur through an increased microbial mass.

Table 3, (page 74) lists three studies (45*-47*) in which the effect of fructooligosaccharides on faecal weight in humans has been measured. The quite limited data indicate an increase in faecal wet weight of around or somewhat more than 1 g/g ingested oligofructose and 1.5–2 g/g ingested inulin. These figures are similar to those reported for pectin (1.2) but considerably lower than for wheat bran (around 5) (44). In a study on elderly constipated patients inulin reduced functional constipation and increased stool frequency (48).

Conclusion: Inulin and fructooligosaccharides seem to have dose-related laxating effects.

Irritable bowel syndrome

Probiotics

Irritable bowel syndrome (IBS) is a very common gastrointestinal disorder, and is often the most frequent diagnosis at a gastroenterologist's clinic. Typical symptoms reported are flatulence, variations between diarrhoea (not in the night) and constipation, and abdominal pains.

Different factors are associated with the condition, e.g. food intake, malabsorption and psychosomatic influences. These factors can exert an effect on the motor function of the gastrointestinal tract. Generally, patients with IBS report pain with a lesser degree of abdominal distention than others. It is always difficult to evaluate self-reported symptoms particularly in a condition with psychosomatic influences like IBS.

In a very carefully performed double-blind RCT study on 61 subjects with IBS, no difference in tolerance was seen with unfermented milk containing *L. acidophilus* compared to ordinary milk (49*).

A rose-hip drink with *L. plantarum* 299v (400 ml/day) was tested in two RCTs with parallel designs. In one of the studies (40 patients), improvement of symptoms was significantly greater in the study group than in the control group (50*). In the other study (52 patients), flatulence was reduced in the test group compared with the placebo group (51*). Abdominal pain was reduced in both groups, even though the reduction was more rapid and pronounced in the test group. There was no major change in gas bloating.

Conclusion: An effect on some symptoms in IBS is reported with *L. plantarum* 299v. More controlled studies are needed for firm conclusions on the importance of probiotics in the treatment of IBS.

Diarrhoea

The effect of probiotics on diarrhoeal disease of varying ætiology has been quite extensively studied and clinical trials have recently been reviewed (52,53). We will concentrate here on four conditions that have been subject to human studies using milk-based products – acute watery diarrhoea in children, travellers' diarrhoea, antibiotic-associated diarrhoea and relapsing diarrhoea due to *Clostridium difficile* infection.

Acute watery diarrhoea in children

Acute diarrhoea in children is mainly caused by rotavirus. *L. rhamnosus* strain GG is the probiotic strain which has been most extensively studied to treat this condition. Both milk products fermented with this bacterium and freeze-dried bacteria have been shown to shorten acute diarrhoea, especially when caused by rotavirus (54*,55*) (Table 2, on pages 71-73). Since acute diarrhoea is self-limiting, seldom lasting for more than a week, the therapeutic effect is small. Thus, the duration of diarrhoea is usually shortened by approximately 1 day (Table 2, on pages 71-73). However, the effect is reasonably well proven and has also been replicated in a number of other studies (for a review see 52,53).

Two studies have investigated the effect of *Lactobacillus reuteri* SD2112 on acute diarrhoea in childhood. Both demonstrate an effect in the same order of magitude as reported for *L. rhamnosus* GG (56*,57*), (Table 2, on pages 71-73).

It should be noted that only one of the above mentioned studies (54*) gives data on the extent of breast-feeding in the different study groups. Since breast-feeding effectively counteracts diarrhoea, it is important to control for this factor.

Conclusion: A significant shortening of acute watery rotavirus-related diarrhoea in children has been demonstrated for both *L. rhamnosus* GG and *L. reuteri* SD2112.

Travellers' diarrhoea

A few placebo-controlled studies have all failed to show effective prevention of infectious diarrhoea in adults (53). These

studies were performed with freeze-dried probiotic preparations, and no data have been published on the possible effects of probiotic food products in this respect.

Conclusion: More controlled trials with probiotic foods are needed and justified since some probiotics have shown an effect on diarrhoea in children.

Antibiotic-associated diarrhoea

Treatment with antibiotics results in diarrhoea and abdominal discomfort in a variable fraction of patients, depending on the age group and the antibiotic used. In most cases, the cause of the diarrhoea is unknown, but a varying proportion of the cases are caused by *Clostridium difficile*. This toxin-producing species is not uncommon in the normal intestinal microflora, but is usually present only in low numbers and without causing any harm. After treatment with certain antibiotics, the lack of competition from other microbes in the normal intestinal flora permits *C. difficile* to reach high numbers. The *C. difficile* toxins may cause anything from mild diarrhoea, which can be cured simply by terminating the antibiotic treatment, to the life-threatening disease pseudomembranous colitis.

Table 2 (on pages 71-74) includes four randomised placebo-controlled studies (58*-61*) that investigate the effect of probiotic intake on gastrointestinal side effects of antibiotic treatment. A yoghurt containing bifidobacteria was shown to quite effectively reduce abdominal complaints in volunteers consuming erythromycin for 3 days (58*). In another study, using parallel groups, no clinical effects of yoghurt with bifidobacteria and *Lactobacillus acidophilus* were noted, but on the other hand, inulin was added to the yoghurt, which in itself may cause loose stools and abdominal discomfort (59*). An interesting observation was that *C. difficile* was isolated from stool cultures of six of ten in the control group, but in only one of nine volunteers in the group given the active yoghurt preparation (p=0.08).

The effect of *Lactobacillus rhamnosus* GG was studied in volunteers taking erythromycin for 7 days (60*). The data are poorly described in the paper, but according to the authors the volunteers given placebo experienced diarrhoea for 8 days and the volunteers given lactobacilli had diarrhoea only for 2 days.

Arvola et al. studied the potential of *L. rhamnosus* GG to reduce the risk of antibiotic-associated diarrhoea in a clinical setting (61*). Children receiving antibiotics against respiratory tract infection (amoxicillin being most frequent, followed by penicillin) were randomised to placebo or capsules with lactobacilli. The drop-out rate was quite high and the therapeutic effect was of borderline significance.

Conclusion: Despite the individual drawbacks of the studies cited above, they offer promising indications that probiotics could be useful against side effects of antibiotic treatment. Larger and better controlled studies with probiotic foods are urgently needed.

Relapsing Clostridium difficile *infection*

Severe *C. difficile* infection is treated with antibiotics active against anaerobic bacteria (vancomycin or metronidazol). The treatment is successful in most cases, but in some 20% of patients, *C. difficile* is not eradicated and the patient is plagued by recurrent episodes of diarrhoea. This condition, termed relapsing *C. difficile* infection, is difficult to treat and new therapeutic alternatives are needed in this patient group.

Only a single placebo-controlled clinical study has been reported on the effects of probiotics on relapsing *C. difficile*-induced disease. This study utilised the yeast *Saccharomyces boulardii* (62*) (Table 2, on pages 71,72,74). The study was designed, performed and evaluated in an excellent manner, which permits conclusions to be drawn with a high degree of certainty. By adding *S. boulardii* to the metronidazol or vancomycin treatment aiming to eradicate *C. difficile,* the risk of the patient relapsing was halved.

L. rhamnosus GG has only been evaluated in open trials against relapsing *C. difficile* disease. Five adult patients were treated with 10^{10} CFU/day, and four experienced no further relapses (63). Four children who were treated with *L. rhamnosus* GG became asymptomatic (64).

Bennet et al. (65*) studied a large series of adult patients, some of whom were referred to a specialist clinician because of relapsing *C. difficile* infection. Some of the patients were residents in a nursing home consistently plagued with *C. difficile* disease. The patients were given capsules containing *L. rhamnosus* GG without addition of antibiotics (65*) (Table 2, on pages 71,72,74). After a single treatment period which lasted for 10–21 days, 84% of the patients did not relapse within the follow-up period, which was 1 month for the ambulatory patients and 2 months for the nursing home patients. Since the trial was open, we do not know what the relapse rate would have been, had the patients not been given probiotics. However, it is reasonable to believe that half of the patients would have relapsed during that period without treatment, based on figures from the large multicentre study of McFarland (62*). The study thus indicates that *L. rhamnosus* could be a promising candidate for treating relapsing *C. difficile* infection.

Conclusion: One probiotic agent, *S. boulardii,* has been convincingly shown to reduce the risk of relapsing with *C. difficile* diarrhoea. For other microorganisms, we have only data from open trials. Since this is a very important potential application for probiotics, controlled studies with probiotic foods should be carried out.

Potential mechanisms involved in control of diarrhoea

It was originally assumed that the ability of probiotic bacteria to shorten diarrhoea was dependent on their ability to colonise the intestine and "alter the microbial balance" in such a way that the pathogen would be eliminated. Specific probiotic strains such as *L. plantarum* 299 and 299v (66), *L. rhamnosus* 271 (66) and *L. rhamnosus* GG (67) have been proven to colonise human volunteers. This might relate to the fact that these lactobacillus species are prevalent on the normal human intestinal mucosa (68). However, some probiotic bacteria do not colonise, but are eliminated at a rate similar to ingested inert particles (69,70). Nevertheless, probiotics that are not likely to colonise can still reduce diarrhoea. This is most strikingly demonstrated for the yeast *S. boulardii* that has been unequivocally proven effective against *C. difficile*-induced symptoms without being able to colonise the intestine. But also bifidobacteria seem to reduce diarrhoea caused by antibiotic treatment (58*) without being able to colonise the individual (59*).

Another proposed effect has been that the probiotic induces an enhanced immune response against the microorganism causing the diarrhoea and that this leads to earlier resolution of the diarrhoeal disease. This has been proposed to be the mechanism of the effect of *L. rhamnosus* GG against rotavirus diarrhoea (71). But differences in antibody titres between patients fed *L. rhamnosus* GG and controls appear only in the convalescence phase (71), when the virus has long since disappeared. Moreover, no differences in antibody titres were seen between patients fed *L. reuteri* and controls (56*), although this organism seemed equally as efficient as *L. rhamnosus* in controlling the diarrhoea (56*,57*).

The mechanism by which probiotic intake reduces diarrhoea

must therefore be regarded as entirely unknown. Potential mechanisms include an influence on the enteric nervous system and/or immune system leading to the production of neuropeptides (72), cytokines (73) or hormones (74) that reduce the secretion of water and electrolytes across the intestinal epithelium.

In the case of *C. difficile* diarrhoea, the probiotic might act on the host, reducing the secretory response to the clostridial toxin, as outlined above. It is also possible that the probiotic changes the milieu in the intestine, leading to reduced toxin production by the clostridiae, since the toxin production of these organisms is strictly controlled by environmental conditions (75).

Gastritis and reflux disease

L. acidophilus strain LA1 was grown in milk and tested against *Helicobacter pylori* induced gastritis in a clinical study with promising effect. Suppression of the infection was determined by a standard breath test (76). However, the effect was reversible, as also seen in a Japanese study with a probiotic *Clostridium* preparation. More recently Canducci et al. (77) tested *L. rhamnosus* GG strain together with antibiotic therapy with fewer gastrointestinal side effects in the probiotic group, suggesting that probiotics could be designed to improve *H. pylori* treatment outcome and prevent side effects such as symptoms of increased acid reflux post-treatment.

Inflammatory bowel disease

Inflammatory bowel disease (IBD) may be caused or aggravated by alterations in the microbial flora. Thus the distal ileum and colon are most frequently affected by the inflammatory process in patients with IBD, sites which harbour the largest populations of intestinal bacteria. Early studies with probiotic lactic acid bacteria (LAB) (*L. reuteri* and *L. plantarum* 299v) strains showed protective effect in chemically induced colitis in rodents. More recently, studies in humans with ulcerative colitis given mesalazine or capsules, containing a well defined *E. coli* strain, showed no diffeence in relapse rates, but unfortunately no placebo group was included.

One placebo-controlled clinical study was performed recently on patients with pouchitis, a common long-term inflammation of the ileal reservoir created after surgical removal of the colon. Patients on this therapy for 9 months showed few relapses (15%) compared to 100% in the control group. This study further supports the potential role of probitics food products in IBD therapy and prophylaxis (78-80).

Recently, a complex probiotic preparation containing 200 billion per gram of viable freeze-dried bacteria of four LAB strains, three bifidobacteria and one strain of *Streptococcus salivarius*, subspecies *thermophilus*, was tested in a clinical trial in patients showing allergy or intolerance of other origin to classical therapy with mesalamine or sulphasalazine (78-80). The treated group showed reduced faecal pH and remained in remission. In another study, the same complex preparation (VSL x 3 from Yovis, Sigma Tau Pomezia, Italy) showed good effects on patients with chronic relapsing pouchitis combined with increased LAB and *S. salivarius* counts in pouch contents (80).

Mechanisms: Recent observations in murine knockout models for IBD suggest that an immunlogical up-regulated Th1 cell response and breaking of the mucosal tolerance against the indigenous gut microflora are involved in various forms of IBD (79). Other recent observations indicate that patients with ulcerative colitis lack a normal indigenous LAB microflora in colonic biopsies, also supporting the hypothesis that IBD may be prevented by replacement by an appropriate pro- and prebiotic food based regime.

Conclusions: Well-designed large-scale randomised placebo-controlled clinical trials of different pro- and prebiotics preparations *versus* standard therapy in IBD and pouchitis should now be undertaken. Promising results of probiotic preparations have been reported and should encourage studies with pro-, pre-, and synbiotic foods.

Cancer prevention

Various enzymes in the gut microflora modify ingested foreign compounds such as nitro aromatics, azocompounds and nitrate, which can be metabolised to genotoxic and carcinogenic substances by enzymes of the anaerobic microflora of the colon (81). A number of studies have shown that diet and antibiotics can change the microflora-associated characteristics, and non-digestible oligosaccharides (NDOs) suppress carcinogen metabolising enzyme activities in rats (82). Furthermore, LAB and bifidobacteria have generally low activities of enzymes involved in carcinogen production. Supplementation of galactooligosaccharides (GOS) and the synthetic disaccharide lactulose have been shown to decrease faecal β-glucuronidases and increase lactobacill counts in rats (83,84).

However, despite these experimental facts, evidence is still missing from human studies that LAB and prebiotics such as GOS and FOS (galactose- and fructooligosaccharides) would decrease the risk of colon cancer development in humans. Two early studies in Japan on the treatment of human urinary bladder cancer by *L. casei* (Yakult) indicates that immunomodulatory effects of LAB and bifidobacteria cells may be used in the future to prevent and treat cancer of the human colon (84,85). However, further studies in this direction should be performed.

Two studies with probiotic milk products in patients undergoing radiotherapy for pelvic malignancies indicate that such products should be further tested to prevent therapy-related diarrhoea and clinical bowel discomfort symptoms (86,87).

Conclusion: Further identification and validation of biomarkers for risk of cancer is a prerequisit for further studies, to evaluate the potential of probiotics and prebiotics in man in relation to cancer.

Prebiotics – mineral absorption

Experiments with rats have shown that non-digestible oligosaccharides like inulin and oligofructose can increase the absorption and retention of minerals such as calcium, magnesium, iron and zink (e.g. 88,89). Similar results have previously been obtained with pectin. From animal studies it is postulated that this enhanced absorption occurs in the colon, and that the mechanism is related to increased solubility of calcium due to lower pH of the colonic content induced by fermentation of oligosaccharides.

Three human studies (Table 4, page 75) testing this hypothesis have been published so far. In the first one Coudray et al. (90*) used conventional balance technique during 28-day periods. Forty grams of inulin (successively introduced to obtain the maximum dose during the last 12 days of the period) increased the apparent absorption of calcium from 21 to 34% and the retention from –10 to +92 mg/day, i.e. by about 100 mg/day. A similar improvement in calcium balance was obtained with sugar beet fibre.

When using a double stable isotope technique, van den Heuvel et al. (91*) did not find any effect on calcium or iron absorption after 9 days with 15 g oligofructose/day in young (20–26 y) male subjects. Absorption was measured during 24 h. In a subsequent study (92*) the group used the same technique in boys (14–16 y), but extended the measurement period to 36 h. An increase in true fractional calcium absorption by 11% was obtained.

It should be noted that calcium balance studies need long study periods and a rigorous control of diets. The negative study is consistent with the hypothesis that increased absorption occurs in the colon, since the calcium absorption was measured during a 24 h-period only. This is also consistent with an ileostomy study by Ellegård et al. (38*) in which oligofructose did not alter the small-intestinal absorption of calcium or other minerals.

Conclusion: Two human short-term studies have confirmed data from animal experiments that oligofructose may increase calcium absorption.

Long-term studies with well-controlled diets are needed to evaluate the potential of prebiotics to contribute to increased bone health.

Safety aspects

Probiotics

Lactic acid bacteria (LAB) are Gram-positive anaerobic aerotolerant non-spore-forming rods and cocci that are indigenous inhabitants of the human gastrointestinal tract, vagina and human skin.

LAB have been isolated in immunosuppressed patients with subacute endocarditis, with increasing frequency in recent years. This indicates that LAB can translocate to the blood in cancer and leukaemia patients or in immunosuppression (93). The findings that most clinical LAB isolates belonges to the rhamnosus-casei group, stimulated an extensive study in Finland recently. The *L. rhamnosus* GG strain could not be isolated from any of the LAB-positive blood cultures (94). This study and the development of animal models such as cytostatic-treated mice, orally fed with LAB strains and other microbes, allow us to simulate the situation in immunosuppressed human cancer patients and new syndromes such as HIV/AIDS (95,96). Such studies should be encouraged for "old and new" milk and other food-based probiotic products with equivalent doses of lactobacilli given daily. Such safety studies are naturally also needed for other biotherapy-based regimens such as multi-strain based probiotics against inflammatory bowel disease and pouchitis (79,80).

Conclusion: As concluded in the review by Marteau (93), infections with lactobacilli occur occasionally in immunosuppressed patients, and this safety aspect should be monitored.

Prebiotics

The Nordic Working Group on Food Toxicology and Risk Evaluation (NNT), which is a body under the Nordic Council of Ministers, recently performed a safety evaluation of fructans, as a project within the Nordic Committee of Senior Officials for Food Issues, co-ordinating Nordic work in the field of foods. Within that committee a Nordic project group was established to draft a manuscript that was finally approved by NNT in September 1999 (97). The group reviewed available toxicity studies in experimental animals as well as reports on adverse effects in humans.

The report concluded, mainly from the 1–3-week human studies available, that adverse effects like flatulence, abdominal pain, bloating, cramps and diarrhoea are unlikely to occur with consumption of 20 g fructooligosaccharides (FOS)/day or less for a person weighting 60 kg. This is in agreement with a previous evaluation by the Scientific Committee on Food of the European Commission (SCF), which defined the no-effect-level for laxative effect in humans to 0.3-0.4 g/kg body-weight.

Conclusion: The overall conclusion of this safety evaluation of oligosaccharides was: "FOS had no significant effects, other than gastrointestinal symptoms at doses 5–40 times higher than the no-effect-level for laxative effects in humans". Although not sufficiently studied, there is a tendency that inulin is better tolerated than FOS.

The scientific documentation of probiotic products sold in Sweden in 2000

As part of the investigation of pro- and prebiotics, the scientific documentation of products on the Swedish market in 2000 was scrutinized. The four relevant Swedish producers and one Finnish producer of probiotic products were contacted by the National Food Administration and asked to provide their scientific documentation, focusing on human studies of health-promoting effects and clinical studies evaluating the usefulness of their products in the treatment or prevention of specific diseases or clinical conditions.

It was emphasised that the focus of this literature survey was on original studies of the product(s) in question. In addition to the studies already identified in our literature search, one study (50) on effects in subjects with irritable bowel syndrome was provided with "submitted" status. The published and ongoing human effect studies on probiotic products need further evaluation as a basis for product-specific physiological claims or health claims. Such evaluations have to be made in relation to the type of claims intended to be used and the target group(s) for the different products.

At present, two products have been classified by the Medicinal Products Agency as "natural remedies" with the indication "traditionally used for the normalization of intestinal flora when temporary gastrointestinal disturbances, e.g. in mild diarrhoea and constipation". It should be noted, however, that the classification as natural remedies has been made on "traditional use" and not based on any evaluation of the product-specific documentation of the products.

Classification as Medical Foods would seem relevant when the product is used as part of a specific medical treatment, e.g. to prevent or treat diarrhoea associated with the use of antibiotics, or as a complication to, for instance, radiological treatment.

Final comments

The main aim of a clinical trial is to evaluate the benefits and risks ascribed to a treatment. The validity is ensured by using a control group for comparison or using a crossover technique. Randomisation and double blinding are necessary.

In the present survey we found the studies to vary greatly in quality, from large-scale carefully designed trials to small studies with several flaws in the design or conclusions. If health claims are to be made for probiotics, strict requirements have to be made concerning the quality of study design.

The food industry that develops pro- and prebiotic products should increase their efforts to develop high quality research and well-designed clinical trials on ordinary food products.This area is of great importance for improving human health.

REFERENCES

References listed in Tables (pages 66-75)

1. Diplock AT, Aggett PJ, Ashwell M, Bornet F, Fern EB, Roberfroid MB: Scientific concepts of functional foods in Europe: Consensus document. Br J Nutr 1999;81 Suppl. 1:S1-S27.
2. Gibson GR, Roberfroid MB: Dietary modulation of the human colonic microbiota:introducing the concept of prebiotics. J Nutr 1995;125:1401-12.
3. Van Loo J et al: Functional food properties of non-digestible oligosaccharides: a consensus report from the ENDO project. Brit J Nutr 1999;81:121-2.
4. Conway P: Prebiotics and human health: The state-of-the-art and future perspectives. Scand J Nutr 2001;45:13-21
5. Wold A: Immune effects of probiotics. Scand J Nutr 2001;45:76-85.
6.* Mustapha A, Jiang T, Savaiano DA: Improvement of lactose digestion by humans following ingestion of unfermented acidophilus milk: influence of bilesensitivity, lactose transport, and acid tolerance of lactobacillus acidophilus. J Dairy Sci 1997;80:1537-45.
7.* Montes RG, Bayless TM, Saavedra JM, Perman JA: Effect of milks

inoculatedwith *Lactobacillus acidophilus* or a yogurt starter culture in lactose-maldigestingchildren. J Dairy Sci 1995;78:1657-64.
8.* Lin MY, Savaiano D, Harladner S: Influence of non-fermented dairy products bacterial starter cultures in lactase maldigestion in humans. J Dairy Sci 1991,74:87-95
9.* Vesa TH, Marteau P, Zidi S, Briet F, Pochart P, Rambaud JC: Digestion and tolerance of lactose from yoghurt and different semi- solid fermented dairy products containing *Lactobacillus acidophilus* and bifidobacteria in lactose maldigesters--is bacterial lactase important? Eur J Clin Nutr 1996;50:730-3.
10.*Shermak MA, Saavedra JM, Jackson TL, Huang SS, Bayless TM, Perman JA: Effect of yogurt on symptoms and kinetics of hydrogen production in lactose-malabsorbing children. Am J Clin Nutr 1995;62:1003-6.
11.*Alm L: Effect of fermentation on lactose, glucose, and galactose content in milk and suitability of fermented milk products for lactose intolerant individuals. J Dairy Sci 1982;65:346-52.
12.*Teuri U, Vapaatalo H, Korpela R: Fructooligosaccharides and lactulose causemore symptoms in lactose maldigesters and subjects with pseudo-hypolactasia than in control lactose digesters. Am J Clin Nutr 2000;71:600-2.
13. Hove.H, Norgaard H, Brobeck Mortensen P: Lactic acid bacteria and the human gastrointestinal tract. Europ J Clin Nutr 1999;53:339-50.
14.*de Roos NM, Schouten G, Katan MB: Yoghurt enriched with *Lactobacillus acidophilus* does not lower blood lipids in healthy men and women with normal to borderline high serum cholesterol levels. EJCN 1999;53:277-80.
15.*Massey LK: Effect of chanching milk and yogurt consumption on human nutrient intake and serum lipoproteins. J Dairy Sci 1984; 67:255-62.
16.*Lin SY, Ayres JW, Winkler W, Sandine WE: Lactobacillus effects on cholesterol: *In vitro* and *in vivo* results. J Dairy Sc 1989;72: 2885-99.
17.*Schaafsma G, Meuling WJ, van Dokkum W, Bouley C: Effects of a milk product, fermented by *Lactobacillus acidophilus* and with fructo-oligo-saccharides added,on blood lipids in male volunteers, J Clin Nutr 1998;52:436-40.
18. Anderson JW, Gilliland SE: Effect of fermented milk (yogurt) containing *Lactobacillus acidophilus* L1 on serum cholesterol in hypercholesterol-emic humans. J Am Coll Nutr 1999;18:43-50
19. McNamara DJ, Lowell AE, Sabò JE: Effect of yoghurt intake on plasma lipid and lipoprotein levels in normolipidaemic subjects. Atheriosclerosis 1989;79:167-81.
20.*Bukowska H, Pieczul-Mroz J, Jastrzebska M, Chelstowski K, Naruszewicz M: Decrease in fibrinogen and LDL-cholesterol levels upon supple-mentation of diet with *Lactobacillus plantarum* in subjects with moderately elevated cholesterol. Atherosclerosis 1998;137:437-8.
21.*Agerholm-Larsen L, Raben A, Haulrik N, Hansen AS, Manders M, Astrup A: Effect of 8 week intake of probiotic milk products on risk factors for cardiovascular diseases. Eur J Clin Nutr 2000;54:288-97.
22.*Agerbaek M, Gerdes LU, Richelsen B: Hypocholesterolaemic effect of a new fermented milk product in healthy middle-aged men. Eur J Clin Nutr 1995;49:346-52.
23.*Richelsen B, Kristensen K, Pedersen SB: Long-term (6 months) effect of a new fermented milk product on the level of plasma lipoproteins – a placebo-controlled and double-blind study. Eur J Clin Nutr 1996;50:811-5.
24.*Bertolami MC, Faludi AA, Batlouni M: Evaluation of the effects of a new fermented milk product (Gaio) on primary hypercholesterolemia. Eur J Clin Nutr 1999;53:97-101.
25.*Andersson H, Bosaeus I, Ellegard L, Grahn E, Tidehag P, Hallmans G, et al: Effects of low-fat milk and fermented low-fat milk on cholesterol absorp-tion and excretion in ileostomy subjects. Eur J Clin Nutr 1995; 49:274-81.
26. Taylor GRJ. and Williams CM:Effects of probiotics and prebiotics on blood lipids Brit J Nutr 1998;80:S225-S230.
27. de Rose NM and Katan MB: Effects of probiotic bacteria on diarrhoea, lipid metabolism, and carcinogenesis: a review of papers published between 1988 and 1998. Am J Clin Nutr 2000;71:405-11.
28. Delzenne NM, Williams CM: Actions of non-digestible carbohydrates on serum lipids in humans and animals. In: Gibson GR, Roberfroid MB (eds). Colonic Microbiota, Nutrition and Health. Kluwer Academic Publishers, Dordrecht/Boston/London, 1999;pp213-31.
29.*Yamashita K, Kawai K, Itakura M: Effects of fructo-oligosaccharides on blood glucose and serum lipids in diabetic subjects. Nutr Res 1984;4:961-6.
30.*Brighenti F, Casiraghi MC, Canzi E, Ferrari A: Effect of consumption of a ready-to-eat breakfast cereal containing inulin on the intestinal milieu and blood lipids in healthy male volunteers. Eur J Clin Nutr 1999;53:726-33.
31.*Canzi E, Brighenti F, Casiraghi MC, Del Puppo E, Ferari A: Prolonged consumption of inulin in ready-to-eat breakfast cereals: Effects on intes-tinal ecosystem, bowel habits and lipid metabolism. Cost 92. *(Abstract)*
32.*Davidson MH, Maki KC, Synecki C, Torri SA, Drennan KB: Effects of dietary inulin on serum lipids in men and women with hypercholesterol-emia. Nutr Res 1998;18:503-17.
33.*Pedersen A, Sandstrom B, Van Amelsvoort JM: The effect of ingestion of inulin on blood lipids and gastrointestinal symptoms in healthy females. Br J Nutr 1997;78:215-22.
34.*Alles MS, de Roos NM, Bakx JC, van de Lisdonk E, Zock PL, Hautvast GA: Consumption of fructooligosaccharides does not favorably affect blood glucose and serum lipid concentrations in patients with type 2 diabetes. Am J Clin Nutr 1999;69:64-9.
35.*van Dokkum W, Wezendonk B, Srikumar TS, van den Heuvel EG: Effect of nondigestible oligosaccharides on large-bowel functions, blood lipid concentrations and glucose absorption in young healthy male subjects. Eur J Clin Nutr 1999;53:1-7.
36.*Luo J, Rizkalla SW, Alamowitch C, Boussairi A, Blayo A, Barry J-L, Laffitte A,Guyon F, Bornet FRJ, Slama G: Chronic consumption of short-chain fructooligosaccharides by healthy subjects decreased basal hepatic glucoseproduction but had no effect on insulin-stimulated glucose metabolism 1-3. Am J Clin Nutr 1996;63:939-45.
37.*Jackson KG, Taylor GR, Clohessy AM, Williams CM: The effect of the daily intake of inulin on fasting lipid and glucose concentrations in middle-aged menand women. Br J Nutr 1999 ;82:23-30.
38.*Ellegård L, Andersson H, Bosaeus I: Inulin and oligofructose do not influence the absorption of cholesterol, or the excretion of cholesterol, Ca, Mg, Zn, Fe, or bile acids but increases energy excretion in ileostomy subjects. Eur J Clin Nutr 1997;51:1-5.
39.*Hata Y, Yamamoto M, Ohni M, Nakajima K, Nakamura Y, Takano T: A placebo-controlled study of the effect of sour milk on blood pressure in hypertensive subjects. J Clin Nutr 1996;64:767-71.
40.*Kawase M: Effect of administration of fermented milk containing whey protein concentrate to rats and healthy men on serum lipids and blood pressure. J Dairy Sci 2000;83:255-62.
41.*Ling WH, Hänninen O, Mykkänen H, Heikura M, Salminen S, Von Wright A: Colonization and fecal enzyme activities after oral Lactobacillus GG administration in elderly nursing home residents. Ann Nutr Metab 1992; 36:162-6.
42. Benno Y. et al: Effects of Lactobacillus GG yoghurt on human intestinal microecology in Japanese subjects. Nutrition Today Supplement 1996; 331:9S-11S.
43.*Alm L, Humble D, Ryd Kjellen E, Setterberg G: The effect of acidophilus milk in the treatment of constipation in hospitalised geriatric patients. In: Nutr and the intestinal flora. XV Symp Swedish Nutrition Foundation Stockholm: Almqvist & Wiksell International; 1983;pp131-8.
44. Cummings JH: The effect of dietary fiber on fecal weight and composition. In: Spiller GA, ed. CRC Handbook of dietary fibre in human nutrition, 2nd ed. Boca Raton, FL:CRC 1993;263-349.
45.*Gibson GR, Beatty ER, Wang X, Cummings JH: Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastro-enterology 1995;108:975-82.
46.*Alles MS, Hautvast JG, Nagengast FM, Hartemink R, Van Laere KM, Jansen JB. Fate of fructo-oligosaccharides in the human intestine. Br J Nutr 1996;76:211-21.
47.*Castiglia-Delavaud C, Verdier E, Besle JM, Vernet J, Boirie Y, Beaufrere B, et al. Net energy value of non-starch polysaccharide isolates (sugar beet fibre and commercial inulin) and their impact on nutrient digestive utilization in healthy human subjects. Br J Nutr 1998;80:343-52.
48. Kleessen B, Sykura B, Zunft H-J, Blaut M: Effects of inulin and lactose on fecalmicroflora, microbial activity, and bowel habit in elderly constipated persons. Am J Clin Nutr 1997;65:1397-1402
49. Newcomer AD, Park HS, O'Brien PC, McGill DB: Response of patients with irritable bowel syndrome and lactase deficiency using unfermented acidophilus milk. Am J Clin Nutr 1983;38:257-63.
50.*Niedzielin K, Kordecki H, Birkenfeld B: A controlled, double-blind, randomised study on the efficacy of *Lactobacillus plantarum* 299v in patients with irritable bowel syndrome. Submitted to the Eur J Gastro-enterol Hepatol 2001 (In press).
51.*Nobaek S, Johansson ML, Molin G, Ahrne S, Jeppsson B: Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome. Am J Gastroenterol 2000; 95:1231-8.
52. Saavedra JM and Abi-Hanna A: Clinical studies of probiotic agents. In: Probiotics, Other Nutritional Factors, and Intestinal Microflora (eds L.Å. Hanson and R.H. Yolken). Linppincott-Raven publ., Philadelphia. Nestlé Nutrition Workshop Scrics 1999;42:47-61.
53. Pathmakanthan S, Meance S, Edwards CA: Probiotics: A revies of human studies to date and methodological approaches. Microb Ecol Health Dis 2000;suppl 2:10-30.
54.*Isolauri E, Juntunen M, Rautanen T, Sillanaukee P, Koivula T. A: Human *Lactobacillus* strain (*Lactobacillus casei* sp strain GG) promotes recovery from acute diarrhea in children. Pediatrics 1991;88:90-7.
55.*Kaila M, Isolauri E, Soppi E, Virtanen E, Laine S, Arvilommi H: Enhan-cement of the circulating antibody secreting cell response in human diarrhea by a human *Lactobacillus* strain. Pediatr Res 1992;32:141-4.
56.*Shornikova AV, Casas IA, Isolauri E, Mykkanen H, Vesikari T: *Lacto-bacillus reuteri* as a therapeutic agent in acute diarrhea in young children. J Pediatr Gastroenterol Nutr 1997;24:399-404.
57.*Shornikova AV, Casas IA, Mykkanen H, Salo E, Vesikari T: Bacterio-therapy with *Lactobacillus reuteri* in rotavirus gastroenteritis. Pediatr Infect Dis J 1997;16:1103-7.
58.*Colombel JF, Cortot A, Neut C, Romond C: Yoghurt with *Bifidobacterium longum* reduces erythromycin-induced gastrointestinal effects. Lancet 1987;2:43.
59.*Orrhage K, Sjostedt S, Nord CE: Effect of supplements with lactic acid bacteria and oligofructose on the intestinal microflora during admi-nistration of cefpodoxime proxetil. J Antimicrob Chemother 2000;46:603-12.
60.*Siitonen S, Vapaatalo H, Salminen S, Gordin A, Saxelin M, Wikberg R, et al: Effect of *Lactobacillus* GG yoghurt in prevention of antibiotic-associated diarrhoea. Ann Med 1990;22:57-9.
61.*Arvola T, Laiho K, Torkkeli S, Mykkanen H, Salminen S, Maunula L, et al.: Prophylactic *Lactobacillus* GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. Pediatrics 1999; 104:64.
62.*McFarland LV, Surawicz CM, Greenberg RN, Fekety R, Elmer GW, Moyer KA, et al: A randomized placebo-controlled trial of *Saccharomyces boulardii* in combination with standard antibiotics for *Clostridium difficile* disease. Jama 1994;271:1913-8.
63. Gorbach SL, Chang TW, Goldin B: Successful treatment of relapsing

Clostridium difficile colitis with *Lactobacillus* GG. Lancet 1987;2:1519.
64. Biller JA, Katz AJ, Flores AF, Buie TM, Gorbach SL: Treatment of recurrent *Clostridium difficile* colitis with *Lactobacillus* GG. J Pediatr Gastroent Nutr 1995;21:224-6.
65.*Bennet RG, Gorbach SL, Goldin BR, Chang T-W, Laughon BE, Greenough IWB, et al: Treatment of relapsing *Clostridium difficile* diarrhea with *Lactobacillus* GG. Nutrition Today 1996;31(6, Suppl 1):35S-38S.
66. Johansson ML, Molin G, Jeppsson B, Nobaek S, Ahrné S, Bengmark S: Administration of different *Lactobacillus* strains in fermented oatmeal soup: in vitro colonization of human intestinal mucosa and effect on the indigenous flora. Appl Environ Microbiol 1993;59:15-20.
67. Alander M, Satokari R, Korpela R, Saxelin M, Vilpponen-Salmela T, Mattila-Sandholm T, von Wright A: Persistence of colonization of human colonic mucosa by a probiotic strain, *Lactobacillus rhamnosus* GG, after oral consumption. Appl Enivon Microbiol 1999;65:351-4.
68. Ahrné S, Nobaek S, Jeppsson B, Adlerberth I, Wold A.E, Molin G: The normal *Lactobacillus* flora of healthy human oral and rectal mucosa. J Appl Microbiol 1998;85:88-94.
69. Vesa T, Pochart P, Marteau P: Pharmacokinetics of *Lactobacillus plantarum* NCIMB 8826, *Lactobacillus fermentum* KLD, and *Lactococcus lactis* MG 1363 in the human gastrointestinal tract. Aliment Pharmacol Ther 2000;14:823-8.
70. Rambaud JC, Bouhnik Y, Marteau P, Pochart P: Manipulation of the human gut microflora. Proc Nutr Soc 1993;52;357-66.
71. Kaila M, Isolauri E, Saxelin M, Arvilommi H, Vesikari T: Viable versus inactivated *Lactobacillus* strain GG in acute rotavirus diarrhoea. Arch Dis Child 1995;72:51-3.
72. Ciancio MJ, Chang EB: Epithelial secretory response to inflammation. Ann N Y Acad Sci 1992;664:210-21.
73. Rocha MFG, Soares AM, Flores CA, Steiner T.S, Lyerly DM, Guerrant RL, Ribeiro RA, Lima AAM: Intestinal secretory factor released by macrophages stimulated with *Clostridium difficile* toxin A: role of interleukin-1b. Infect Immun 1998;66:4910-6.
74. Johansson E, Lönnrot I, Lange S et al: Molecular cloning and expression of a pituitary gland protein modulating intestinal fluid secretion. J Biol Chem 1995;270:20615-20.
75. Karlsson S, Lindberg A, Norin E, Burman LG, Åkerlund T: Toxins, butyric acid, and other short chain fatty acidsare coordinately expressed and downregulated by cysteine in *Clostridium difficile*. Infect Immun 2000;68:5881-8.
76. Michetti P, Dorta G, Wisel PH, Brassart D, Verdu E, Herranz M, Felley C, Porta N, Rouvet M, Blum AL, Corthésy-Theulaz I: Effect of whey-based culture supernatant of *Lactobacillus acidophilus* (*johnsonii*) La1 on *Helicobacter pylori* Infection in humans Digestion 1999;60:203-209.
77. Canducci F et al: A lyophilized and inactivated culture of *Lactobacillus acidophilus* increases *Helicobacter pylori* eradication rates. Aliment Pharmacol Ther 2000;14:1625-9.
78. Gionchetti P, Rizzello F, Venturi A, Brigidi, P, Matteuzzi D, Bazzocchi G, Poggioli G, Miglioli M, Campieri M: Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: A double-blind, placebo-controlled trial. Gastroenterology 2000;119:305-9.
79. Xavier RJ, Podolsky DK: How to get along – friendly microbes in a hostile world. Science 2000;289;1483-4.
80. Campieri M, Gionchetti P: Probiotics in inflammatory bowel disease. New insight to pathogenesis or a possible therapeutic alternative? Gastroenterology 1999;116:1246-60.
81. Burns AJ, Rowland JR: Anti-carcinogenicity of probiotics and prebiotics. Curr Issues Intestinal Microbiol 2000;1:13-24 .
82. Hirayama K, Rafter J: The role of probiotic bacteria in cancer prevention. Microbes Infect 2000;2:681-6. .
83. Hosoda M, Hashimoto H, He D, Morita H, Hosono A: Effect of administration of milk fermented with *Lactobacillus acidophilus* LA-2 on fecal mutagenicity and microflora in the human intestine. J Dairy Sci 1996;79: 745-9.
84. Aso Y, Akazan H: Prophylactic effect of a *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer. Urol Int 1992;49:125-9.
85. Aso Y, Akaza H, Kotake T, Tsukamoto T, Imai K, Naito S: Preventive effect of a *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer in a double-blind trial. The BLP Study Group. Eur Urol 1995; 27: 104-9.
86. Henriksson R, Franzén L, Sandström K, Nordin A, Arevärn M, Grahn E: Effects of active addition of bacterial cultures in fermented milk to patients with chronic bowel discomfort following irradiation. Support Care Cancer 1995;3:81-3.
87. Salminen E, Elomaa, I, Minkkinen J, Vapaatalo H, Salminen S: Preservation of intestinal integrity during radiotherapy using live *Lactobacillus acidophilus* cultures. Clin Radiol 1988;39:435-7.
88. Otha A, Ohtsuki M, Baba S, Takizawa T, Adachi T, Kimura S: Effect of fructooligosaccharides on the absorption of iron, calcium, and magnesium in iron deficient anemic rats. J Nutr Sci Vitaminology 1995;41:281-91.
89. Scholz-Ahrens K, Van Loo J, Schrezenmeyer J: Oligofructose stimuliert die Femurmineralisation in Abhängigkeit von der Calciumzufuhr bei der ovariektomisierten Ratte (The increase in bone mineralization in the ovarectomized rat by oligofructose also depends on Ca supplementation). Zeitschrift für Ernährungswissenschaft 1998;37:123-4.
90.*Coudray C, Bellanger J, Castiglia-Delavaud C, Remesy C, Vermorel M, Rayssignuier Y: Effect of soluble or partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. Eur J Clin Nutr 1997;51:375-80.
91.*van den Heuvel EG, Schaafsma G, Muys T, van Dokkum W: Nondigestible oligosaccharides do not interfere with calcium and nonheme-iron absorption in young, healthy men. Am J Clin Nutr 1998;67:445-51.
92.*van den Heuvel EGHM, Muys T, Van Dokkum W, Schaafsma G: Oligofructose stimulates calcium absorption in adolescents. Am J Clin Nutr 1999;69:544-8.
93. Marteau P: Safety aspects of probiotic products. Scand J Nutr 2001;45:22-4.
94. Saxelin M, Chuang NH, Chassy B, Rautelin H, Mäkelä PH, Salminen S, Gorbach SL: Lactobacilli and bacteriemia in Southern Finland, 1989-1992. Clin Infect Dis 1996;22:564-6.
95. Antony, Suresh J: Lactobacillemia: An emerging cause of infection in both the immunocompromised and the immunocompetent host. J Natl Med Assoc 2000;92:83-6.
96. Fruchart C, Salah A, Gray C, Martin E, Stamatoulla A, Bonmarchand G, Lemeland IF, Tilly H: *Lactobacillus* species as emerging pathogens in neutropenic patients. Eur Clin Microbiol Infect Dis 1997;16:681-4.
97. Anon: Safety evaluation of fructans.Nordic Council of Ministers, Copenhagen 2000; TemaNord 2000:523;pp115.

The article continues with Tables 1-4.

Table 1.Studies on lactose intolerance, blood lipids, hypertension and irritable bowel syndrome, columns 1-6 *(cont).*

1. Ref.	2. Main hypothesis	3. Intervention method	4. Study design	5. Recruitment/ Inclusion criteria	6. Exclusion criteria
Mustapha et al (6)	Improvement of lactose digestion with unfermented acidophilus milk	Acidophilus test milks	RCT, double-blind	11 maldigesting subjects	
Montes et al (7)	Reduction of symptoms and reduced H_2 excretion with a yoghurt culture	250 ml milk with *L. acidophilus* or yoghurt	CT	20 children with low lactase activity	
Lin et al (8)	*L. acidophilus* reduces breath hydrogen values	1. 400 ml milk 2. 400 ml acidophilus milk 3. 400 ml yoghurt	CT		
Vesa et al (9)	Different digestibility and tolerance of lactose from products with different lactase contents and bacterial cultures	10 g of lactuloses and 18 g of lactose in 1. Youghurt 2. Milk with *L. acidophilus* and *bifidobact.* 3. Milk with *L. bulgaricus*	Metabolic ward study RCT and cross-over	Lactase-deficient healthy subjects	
Shermakk et al (10)	Yoghurt improves lactose malabsorption and symptoms in children	1 12 g lactose in milk 2 12 g lactose in yoghurt	CT	14 children	
Alm (11)	Acidophilus or yoghurt do not give symptoms in lactose intolerant subjects	500 ml yoghurt 500 ml acidophilus 500 ml milk	Trial	Lactose intolerant subjects controls	
Teuri et al (12)	Fructo-oligosaccharides cause symptoms in lactose maldigesters	1. 50 g lactose 2. 50 g sucrose 3. 25 g lactose 4. 25 g fructooligo-saccharides	CT		
De Roos et al (14)	*L. acidophilus* strain L-1 lowers serum cholesterol	500 ml of yoghurt 1. with and 2 without *L. acidophilus*	RCT Parallel trial	Healthy free-living subjects	Serum cholesterol more than 7.8 mmol/l
Massey (15)	Milk and yoghurt reduces lipoproteins	1. a) 1500 ml milk b) no milk c) 1250 ml nonfat milk 2. a) 480 ml low fat yoghurt 3. b) no yoghurt	CT	1. 32 healthy men 2. 30 healthy women	
Lin et al (16)	*L. acidophilus* and *bulgaricus* lower serum cholesterol	1. Tablets containing about x 10^6 viable bacteria of *Lactobacillus* 2. placebo (dead bacteria)	1.*In vitro* study 2.RCT, cross-over	1 Normal subjects 2 Medium cholesterol population 3 High cholesterol population 4 Regular exercise population	-
Schaafsma et al (17)	*L. acidophilus* reduces serum cholesterol	1. 125 ml of a product with *L. acidophilus* 2. 125 ml of traditional yoghurt	RCT, cross-over	Healthy subjects	
Anderson et al (18)	Fermented milk containing *L. acidophilus* reduces serum cholesterol in hypercholesterolemic subjects		RCT		
Bukowska et al (20)	*L. plantarum* 299v lowers serum cholesterol	1. 200 ml Pro Viva 1. 200 ml rose-hip drink	RT, Parallel design	Healthy subjects	Cardiovascular disease,diabetes or hypertention
Agerholm-Larsen et al (21)	GAIO (*Enterococcus faecium* and *Streptococcus thermophilus*) lowers serum cholesterol	450 ml 1. yoghurt (s.t. and e.f.) 2. placebo yoghurt 3. yoghurt, 2 other strains 4. GAIO	RCT Parallel test	Healthy overweight women	
Agerbaek et al (22)	GAIO lowers serum cholesterol	200 ml 1. GAIO 2. placebo GAIO	RCT	Healthy weight stable	
Rickelsen et al (23)	GAIO lowers serum cholesterol after 6 months	200 ml of 1. GAIO 2. Placebo GAIO	RCT	Healthy subjects	
Bertolami et al (24)	GAIO lowers serum cholesterol	1. 200ml GAIO or 2. 200 ml placebo GAIO	RTC	Healthy subjects	
Andersson et al (25)	Reduced cholesterol absorption and increased cholesterol or bile acid excretion with Verum Hälsofil	1. 1000 ml low-fat milk 2. 1000 ml Verum Hälsofil 3. 1000 ml of lemonade	RCT Constant diet	Ileostomy subjects	High bile acid excretion
Yamashita et al (29)	Fructooligosaccarides reduces serum cholesterol	1. 8 g per day of fructooligosaccarides 2. 5 g per day of sucrose	RCT no cross-over	Subjects with diabetes	

cont.

Table 1.Studies on lactose intolerance, blood lipids, hypertension and irritable bowel syndrome, columns 1-6 *(cont).*

Brighenti et al (30)	Inulin reduces serum cholesterol	1. Placebo 2. Placebo + Inulin 3. Habitual diet	RCT	Healthy male volonteers	
Canzi et al (31)	Inulin lowers serum cholesterol	1. 50 g of cereals 2. 50 g of cereals , 3. 9 g inulin	CT	Healthy subjects	
Davidson et al (32)	Inulin reduces serum cholesterol	1. 18 g inulin /d 2 No inulin	RCT, cross-over	Men and women with hypercholesterolemia	
Pedersen et al (33)	Inulin lowers serum cholesterol	1. 14 g of inulin in 40 g margarine 2. 40 g margarine	RCT	Normolipidemic women	
Alles et al (34)	Fructooligosaccarides reduces serum cholesterol	1. 15 g fructo-oligosacharides 2. 4 g placebo	CRT	Type 2 diabetes	
van Doccum et al (35)	Oligosaccarides reduces serum lipids	1. Inulin 2. Fructo-oligosaccarides 3. Galacto-oligosaccarides 4. Control	R double-blind,diet controlled	Healthy men	
Luo et al (36)	Fructooligosaccharides (FOS) influences glucose and lipid metabolism	1. 20 FOS 2. Placebo	CRT	Type 2 diabetes	
Jackson et al (37)	Inulin reduces lipids and glucose	1. 10 g inulin 2. Placebo	CT	Healthy men and women	
Ellegård et al (38)	Inulin lowers serum cholesterol and influences mineral absorption	1. 17 g inulin 2. 17 g oligofructos 3. 7 g sacrose (placebo)	RCT Constant diet	Ileostomy subjects	
Hata et al (39)	Sour milk reduces blood pressure in hypertensive subjects	1. 95 ml Calpis, sour milk 2. 95 ml placebo-milk	RCT, parallel design	Hypertensive subjects	
Kawase et al (40)	Fermented milk reduces serum lipids and blood pressure	1. 200 ml fermented milk 2. Placebo	CT		
Ling et al (41)	Colonization of faeces by *Lactobacillus* GG and effects on bowel function	1. 200 ml placebo 2. 200 ml *Lactobacillus* 3. 200 placebo	CT		
Alm (43)	Acidophilus milk alleviates constipation		Cross-sectional or cross-over study	Constipated subjects from a geriatric ward	
Newcomer et al (49)	Symptoms would be reduced in IBS and lactase-deficient subjects (using acidophilus milk)	1. 240 ml x 3 of ordinary milk 2. 240 ml x 3 of acidophilus milk	Double-blind RCT	1. IBS (61) 2. Lactase-deficient (18) 3. Healthy sbjects (10)	-
Niedzielin et al (50)	Reduction of symptoms	1. Pro Viva 2. Placebo	RT, parallel test	IBS	Inflammatory bowel disease
Nobaek et al. (51)	*L. plantarum* DSM 9843 reduces abdominal bloating and pain	3. 400 ml of a rose-hip drink 4. 400 ml of rose-hip drink containing *L. plantarum*,5 x 10^7 CFU and 0.009 g/ml of oat flour	RCT	Patients with irritable bowel syndrome (IBS)	Malabsorption or patients less than 18 years old.

cont.

Table 1.Studies on lactose intolerance, blood lipids, hypertension and irritable bowel syndrome, columns 7-10 ***(cont).***

Ref.	7. Matching of groups	8. Treatment time	9. Follow up after treatment (if any, e.g. persistence of probiotic organism in faeces, recurrence of symptoms)	10. Number of subjects/patients		
				Number starting	**Number ending experimental period**	**Number followed up** (if any)
6	Cross over	hours		11		
7	No cross-over	hours		20 children		
8	Same subjects					
9	Own controls	8 hours x 4	3-15 days wash-out	15		14
10	No cross-over	8 hours		14 children		
11	Same subjects	hours		8		
12	Cross-over	8 hours		40		
14	Stratification for sex, age, s-cholesterol	6 weeks		85		78 (2 x 39)
15	Cross-over	3-4 weeks		32 + 30		
16	Cross-over study	2 x 6 weeks		Test/control 157 / 177		
17	Cross-over	2 x 3 weeks, wash out		30		30
18	Cross-over	4 weeks+2 weeks + 4 weeks				
20	No cross- over	6 weeks		30		30
21	Matched for sex, age BMI, HDL, LDL	8 weeks		73		70
22	Treatment group 0.21 mmol higher	6 weeks		58		29 GAIO 28 placebo
23	Randomised, good comparison	6 months		90		87
24	Cross-over	2 x 8 weeks		32		32
25	Same subjects, cross-over	3 x 3 days		9		9
29	18 treated 10 controls	2 weeks		18/10		18/10
30	Cross-over	3 x 4 weeks		12		
31	Same subjects	2 x 4 weeks		12		12
32	Cross-over	2 x 3 weeks, wash out		21		21
33	Cross-over	2 x 4 weeks		72		64
34	Cross-over	20 days + 20 days		20		
35	Cross-over	4 x 3 weeks		12		
36	Cross-over	2 x 4 weeks		10		
37	Parallel study	8 weeks		54		
38	Same subjects, cross-over	3 x 3 days		10		10
39	Randomisation	8 weeks		30		
40	Cross-over	8 weeks				
41	Same subjects	Three two weeks periods (baseline, test, baseline)		12		6
43	Same subjects	36-105 days		50		
49	Own controls	2 + 2 weeks	2 weeks wash out	89		61
50	Rand for age, gender	4 weeks		20 + 20		20 + 20
51	Randomized into two groups	4 weeks		30 + 30		25 + 27

cont.

Table 1.Studies on lactose intolerance, blood lipids, hypertension and irritable bowel syndrome, columns 11-14 *(cont).*

Ref.	11. Results						12. a. Side effects b. Compliance measure	13. Evaluation of quality	14. Concluding remarks – strength of evidence
	Main outcome		Other effects		Drop-outs				
	Treatment	Control	Treatment	Control	Treat-ment	Con-trol			
6	Reduced total H_2 production							+	Acidophilus milk improves lactose tolerance and digestion
7	Reduction of symptoms and H_2 with a yoghurt culture							+	Less symptoms with yoghurt
8	Yoghurt delayed breath H_2 peak and gave lower values than in the control							+	Only one strain of LA-1 significantly decreased breath hydrogen values
9	No difference in breath H_2 concentrations after the three test products, no diffenence in symptom scores	Lactulose and lactose gave higher scores			1		12/14 had symptoms	++	Despite the difference in lactase and bacterial content, lactose was as well digested and tolerated from the three different fermented dairy products
10	Lactose absorbed better from yoghurt than milk							+	Less symptoms with yoghurt
11	Milk induced more symptoms than yoghurt and acidophilus in non-Swedish subjects							++	Less amount of lactose gives less symptoms in lactose intolerant subjects
12	More symptoms with undigestible carbohydrates							++	More symptoms with oligo-saccharides in low lactase activity subjects
14	S-cholesterol reduced by 0.02 mml/l	S-cholesterol reduced by 0.07 mmol/ l	Unaffected levels of LDL, HDL and triacyl-glycerol		6 in all		Abdominal symptoms in one subject	+++	Addition of L. acidophilus does not lower serum cholesterol
15	No effects on serum lipids							+++	No effects on serum lipids
16	No effect on total cholesterol, HDL or triglycerides	No effect					6-21% side effects with lactobacillus, 7-15% side effects with placebo	+++	No effect on serum lipids.
17	LDL down: 5,4%							++	Small reduction of LDL
18	Serum cholesterol down by 2.9%	No effect						++	Small effect on serum cholesterol
20	s-cholesterol 233±36 reduced to 216±33 ($p<0.05$)	s-cholesterol 216±31 reduced to 208±40	Fibrogen no change					++	Small reduction in relation to the control group
21	Cholesterol decreased by 8.4%	No sign difference	Fibrinogen increased by 0.7 mmol/l		3		Compliance measured Constipation in two	+++	Mass-sign
22	Cholesterol reduction by 0.37 mmol/l	Cholesterol reduction by 0.02 mmol/l	Trigly-cerides no difference					++	Small reduction in total serum cholesterol
23	Cholesterol down 0.32 after 3 months, after 6 months no difference compared to control group	After 6 months the same reduction as in test group	Trigly-cerides and HDL no change	Trigly-cerides and HDL no change	3			+++	No difference between groups after 6 months. Milk products may have a hypocholesterolemic factor
24	Serum cholesterol down by 5.3% LDL down by 6.15%	HDL up					Insignificant	+++	Small but sign. reduction of s-cholesterol A few subjects showed an increment

cont.

Table 1.Studies on lactose intolerance, blood lipids, hypertension and irritable bowel syndrome, columns 11-14 *(cont).*

25	Lower cholesterol absorption with Verum hälsofil		Highest endogenous cholesterol excretion with low fat milk					+++	No difference in net cholesterol excretion-lower absorption with the Verum product (assimilation of cholesterol by bacteria ?) A hypocholesterolemic factor in milk?
29	Serum cholesterol reduced by 17 mg/dl	No change						+	Small reduction
30	Total cholesterol down by 7.9%	Less reduction	Triglycerides down by 7.8%					+	Inulin seem to have a lipid lowering potential
31	Total cholesterol down to 150 mg/l	Total cholesterol down to 158 mg/dl	TG 54,4 ± 4.4	TG 74.8 ± 7.4				++	Small study TG down
32	Total-cholesterol declined by 1.3% and LDL by 2.%	Total cholesterol increased: 7.4% and LDL cholesterol by 12.3%	Triglycerides no difference					++	"Reduction" due to an increase in the control group
33	TC, LDL, HDL no change	TC LDL,HDL no change	Triglycerides no change		6		Flatulence, cramps	+++	No effect on serum cholesterol of inulin
34	No effect onTC, LDL-cholesterol or triglycerides							+++	Lack of effect not due to insufficient statistical power
35	No sign. difference							++	Lack of effect
36	Total cholesterol unchanged		Glucose metabolism unchanged					+	No effect on glucose and lipid metabolism.
37	Inulin reduces triacylglycerol levels (p<0,08)							++	Trend for a reduction of plasma TAG
38	No difference in cholesterol absorption		No difference in mineral absorption of the small bowel					++	No change in cholesterol metabolism induced by inulin. No change in small bowel mineral absorption
39	Blood pressure down, syst. 14.1 mm Hg. Diast. by 6.9 after 8 wk	No sign change						++	Blood pressure down,small group, parallel study
40	HDL increased after 4 weeks. Triglycerides down. Systolic blood pressure was reduced							++	Changes in serum lipids. Lower blood pressure
41	Colonization of faeces by lactobacilli. No changes in fecal frequency weight or pH		Bowel frequency increased in 7 of 10 patients to 8 or more /week					+	No changes in bowel frequency
43	Reduction in the need for laxatives		A tendency toward increased defecation frequency					++	Non-conclusive results on the effect on bowel evacuation frequency
49	No difference between test groups and controls in diarrhoea, bloating, number of stools and abdominal pains							+++	No difference in the degree of tolerance to the two varieties of milk
50	Reduction of abdominal pain normalisation of stool frequency							++	Effect on symtoms
51	Reduction in flatulence in 44%, reduction in pain by 36%	Reduction in flatulence by 18%,reduction in pain by 18%	No. of days with normal/ hard stools didn´t differ between test and placebo group		5	3		++	Decrease pain and flatulence but does not change the no of days with hard or normal stools on the different regimens between groups

cont.

Table 2.Studies on diarrhoea, columns 1-6 *(cont).*

1. Ref.	2. Main hypothesis	3. Intervention method	4. Study design	5. Recruitment/ Inclusion criteria	6. Exclusion criteria
Isolauri et al (54)	Administration of *L. rhamnosus* GG promotes recovery from acute diarrhoea	1) *L. rhamnosus* GG in 125 g fermented milk product twice daily ($2x10^{10}$-$2x10^{11}$ CFU/d) 2) *L. rhamnosus* GG freeze-dried, same amount 3) Pasteurized yoghurt	RCT	4-45 months Acute diarrhoea of less than 7 days' duration >3 watery stools last 24 h 82% were positive for rotavirus	
Kaila et al (55)	Intake of *L rhamnosus* GG shortens diarrhoea due to rotavirus infection	1) 125 g x 2 per day of milk product fermented with *L. rhamnosus* GG 2) Fermented milk as above, but pasteurized	RCT	Children with acute gastroenteritis of <7 days' duration admitted to hospital during rotavirus epidemic	
Shornikova et al (56)	*L. reuteri* shortens duration of acute diarrhoea in children	1) 10^{10}-10^{11} CFU/d of *L. reuteri* SD2112 2) Placebo (milk powder)	RCT Double-blind	6-36 months of age presenting with acute diarrhoea (1 or more watery stool/d) of less than 7 days' duration during rotavirus season	Not previously fed cow's milk
Sornikova et al (57)	*L. reuteri* shortens duration of rotavirus-induced acute diarrhoea in children	1) 10^{10}-10^{11} CFU *L. reuteri*/d (in capsules with lactose) 2) 10^{7} CFU *L. reuteri*/d (in capsules with lactose) 3) Placebo (capsules with lactose only)	RCT Double-blind	6-36 months of age presenting with acute diarrhoea of less than 7 days' duration and 3 or more watery stools per day during rotavirus season.	Diarrhoea due to other causes than rotavirus and patients requiring i.v. fluid treatment.
Colombel et al (58)	*B. longum*-containing yoghurt prevents gastrointestinal side effects of erythromycin treatment	1) 3 yoghurt servings per day with *B. longum* 2) 3 placebo yoghurt servings per day All were given 1 g erythromycin sulphate for 3 days	RCT Double-blind Cross-over	Healthy men and women (5+5), mean age 29 years	
Orrhage et al (59)	Milk fermented with *B. longum* and *L. acidophilus* prevents *C. difficile* colonization and gastrointestinal side effects cased by antibiotic treatment	1) 250 g fermented milk (*L. bulgaricus* 10^{7-8} CFU/ml, *S. thermophilus* 10^{8-8} CFU/ml) with *B. longum* (1-5 x 10^{10} CFU/d) and *L. acidophilus* (5-7 x 10^{10} CFU/d). 15 g oligofructose added 2) Fermented milk with 15 g oligofructose 3) Fermented milk All:100 mg cefpodoxime proxetil	RCT Double-blind	Healthy women and men age 21-50	Antibiotics last 3 months
Siitonen et al (60)	*L. rhamnosus* GG prevents diarrhoea caused by antibiotic treatment (erythromycin)	1) 125 ml fermented yoghurt twice daily containing *L. rhamnosus* GG 2) 125 ml pasteurized yoghurt twice daily All were given erythromycin acistate 400 mg for 7 days	RCT	Male healthy volunteers 18-24	Antibiotic treatment last 2 months
Arvola et al (61)	Ingestion of *L. rhamnosus* GG during antibiotic treatment prevents gastrointestinal side effects	1) Capsules with $2x10^{10}$ CFU *L. rhamnosus* GG twice daily 2) Placebo capsules during antimicrobial treatment	RCT	Children receiving antibiotics against respiratory tract infections 2w-13 years of age	1) Antimicrobial medication last 3 m 2) Gastrointestinal disorders 3) Intravenous antibiotic treatment
McFarland et al (62)	*Saccharomyces boulardii* increases efficacy of treatment with vancomycin or metronidazol to prevent *C. difficile* recurrence	1) 1 g/d of lyophilized *S. boulardii* in capsules ($3x10^{10}$ CFU/d) 2) Placebo of identical appearance and odour (not further specified)	RCT Double-blind Multicenter	*C. difficile* disease, ranging from uncomplicated diarrhoea to pseudomembranous colitis. Positive *C. difficile* cultures	Inflammatory bowel disease, AIDS, cancer chemotherapy, anti-fungal therapy, pregnancy allergy to vancomycin/ metronidazol, unreliability
Bennet et al (65)	*L. rhamnosus* GG prevents relapse of *C. difficile* diarrhoea	1) 1, 2 or 4 capsules with $2x10^{10}$ CFU *L. rhamnosus* GG	OT	Outpatients referred because of relapsing *C. difficile* diarrhoea, or residents at a nursing home with relapsing *C. difficile* diarrhoea *C. diffficile* diarrhoea: >1 watery stool per day for >2 consecutive days + positive stool culture or toxin test. Relapse: new episode within 4 weeks after treatment	Other likely cause for diarrhoea

cont

Table 2.Studies on diarrhoea, columns 7-10 *(cont).*

Ref.	7. Matching of groups	8. Treatment time	9. Follow up after treatment	10. Number of subjects/patients		
				Number starting	**Number ending experimental period**	**Number followed up** (if any)
54.	92% had rotavirus in group 1, 74% in group 2 and 79% in placebo group Two breastfed infants in groups 1 and 3. Four breast-fed infants in group 2	5 days	4 weeks			1) 24 2) 23 3) 24
55.	Study group 3 months older (17.5 vs 14.3 mo, p=0.09). Clinical picture similar with respect to weight, dehydration, and acidosis.	5 days				1) 22 2) 17
56.	Vomiting more frequent in placebo group (76%) than treatment group before start of the study. Dehydration more common in *L. reuteri* group (p=0.02). Rotavirus-positive cultures from 63% of the patients in the *L. reuteri* group compared with 86% in the placebo group.	Until discharge, maximally 5 days	Four weeks after treatment: rotavirus titres. No difference between groups	1) 19 2) 22	1) 19 2) 21 One in placebo group excluded because of presence of *L. reuteri* in faeces	?
57.		Until discharge, maximally 5 days		97 of which 89% were rotavirus positive = 86. After exclusion of those getting i.v. fluid, 66 remained	1) 21 2) 20 3) 25	
59.		21 d		1) 10 2) 10 3) 10	1) 9 2) 10 3) 10	
60.		7 days		16 (8+8)	16	
61.	Age: 4.7 years (2w-12y) in GG group, compared to 4.4 y (2w-13 y) in control group.	During antimicrobial therapy		167		119
62.		4 weeks	4 weeks Daily diary for stool frequency and consistence, other symptoms and adverse reactions. Weekly telephone interviews.	124	104	95
65.		10 days (first 12 ambulatory patients) 21 days (next 11 patients) 14 days (nursing home patients)	1 month (ambulatory patients) 2 months (nursing home patients	23 ambulatory (14 women, 9 men) 9 nursing home (8 women, 1 man)	32	32

cont.

Table 2.Studies on diarrhoea, columns 11-14 ***(cont).***

Ref	11. Results						12. a. Side effects b. Compliance measure	13. Evaluation of quality	14. Concluding remarks – strength of evidence
	Main outcome		Other effects		Drop-outs				
	Treatment	Control	Treatment	Control	Treat-ment	Control			
54.	Duration of diarrhoea: 1) 1.4 ± 0.8 days 2) 1.4 ± 0.8 days 3) 2.4 ± 1.1 days (p<0.001, ANOVA) In rotavirus-positive cases: 1) 1.4 (0.8) 2) 1.4 (0.9) 3) 2.7 (1.0) (p<0.001, ANOVA)		No difference in vomiting between groups. No difference in mannitol secretion between groups.					++(+)	++(+) Ingestion of milk product fermented with *L. rhamnosus* GG or freeze-dried bacteria shortens acute diarrhoea in children (mainly rotavirus-induced)
55.	Diarrhoea day 3: 9% Duration of diarrhoea: 1.1 day	53% p=0.002 2.5 days p=0.001						++(+) No data on breast-feeding	++(+) Intake of milk product fermented with *L. rhamnosus* GG shortens rotarivus-induced diarrhoea in children. Small effect, but well proven
56.	Diarrhoea prevalence: day 1: 84% day 2: 26% day 3: 11% Mean duration of diarrhoea shorter in *L. reuteri* group (p=0.07)	 100% p=0.06 81% p<0.001 53% p<0.01	Vomiting reduced in *L. reuteri* group d 3 (p=0.04).				Stool cultures day 0, 2 and at discharge. *L reuteri* present in treatment, but not control group	++ No data on breast-feeding Faeces not cultured for any pathogens	++ *L. reuteri* shortens duration of acute diarrhoea in children
57.	Diarrhoea prevalence day 1: 1) 81% (p=0.01 cmp with group 3) 2) 100% 3) 100% Diarrhoea day 2: 1) 48% (p=0.04 cmp with group 3) 2) 70% 3) 80%		Mean duration of diarrhoea: 1) 2.5 (1.5) d 2) 1.9 (0.9) d 3) 1.5 (1.0) d *L. reuteri* had sign. effect on duration of diarrhoea (p=0.01, ANOVA)				Stool cultures from 40 patients. *L. reuteri* counts: 1) 10^{48} CFU/g 2) 10^{27} CFU/g $<10^{1.7}$ CFU/g	++(+) No breast-feeding data	++(+) *L. reuteri* shortens acute rotavirus-induced diarrhoea in children
58.	Stool weight increase (day 1-3): 98 g/d Abdominal discomfort: 1/10 Clostridial spores: 1/10	 13 g/d 6/10 7/10	Sign. lower (p=0.025, paired test) increase in faecal weight and stool number during period with BA yoghurt					++ Small study, but elegant design and clear-cut significances	++ Yoghurt with bifidobacteria reduces diarrhoea and abdominal complaints after erythromycin treatment ++ Significantly (p=0.02, Fisher's exact test) lower carriage rate of clostridial spores
59.	1) 1/9 colonized by *C. difficile* 2) 6/10 " 3) 6/10 " 1) 5/9 loose stools, 0/9 constipated 2) 6/10 " , 2/10 " 1) 2/10 " , 3/10 "				1 in group 1, due to treat-ment with other anti-biotics			++ small study	+ No significant changes in clinical parameters. Possible reduction of *C. difficile* cololnization (p=0.08 Fisher's test)

cont.

Table 2.Studies on diarrhoea, columns 11-14 *(cont).*

60.	Duration of diarrhoea: 2 days Stomach pain: 23%	 8 days p<0.05 39% p=?	Reduced abdominal distress, pain and flatulence				Blood concentration of erythromycin measured on 1st and last day Stool samples 1st and last days. *L. rhamnosus* GG identified by morphology	+(+) Primary data on diarrhoeal frequency, duration not given in publication Small study	+ Reduced diarrhoea in GG group, but insufficient data given to judge strength of conclusion
61.	Diarrhoea (>2 watery stools/d for >1d) 3 (5%) No causative agent found in most cases of diarrhea	 9 (16%) p=0.05	*C. difficile*-positive individuals: 1	 1	28	20	Faecal cultures were screened for *L. rhamnosus* GG in 23 randomly selected patients. 21/23 had >10e3 GFU/g faeces	++ relatively large study, but large drop-out rate. No drop-out analysis	+(+) *L. rhamnosus* GG reduces incidence of antibiotic-associated diarrhoea. Effect not quite significant and large drop-out.
62.	*C. difficile* recurrence: 26.3% No of stools/d: 2.1 Toxin B in faeces: 6.7% No effect on pain, nausea, or cramps.	 44.8% p=0.05 3.3 p=0.02 30% p=0.02	4 deaths (*S. aureus* sepsis, resp. arrest, cardiac arrest, prostate cancer)	1 death (pneumonia)	Calculations based on intention-to-treat		a) Increased thirst: p=0.02 b) Comparison of patient diary with number of capsules returned by end of study	+++ Excellent study Statistics based on intention-to-treat. Relapse of *C. difficile* infection strictly defined and assessed by three independent, blinded observers	+++ *Saccharomyces boulardi* potentiated treatment with vancomycin or metronidazol in preventing *C. difficile* recurrence
65.	84% did not relapse during follow-up period							++ relatively large patient group. No placebo group.	+(+) *L. rhamnosus* GG seems to be effective against relapsing *C. difficile*, but controlled study needed

Table 3. Studies in which the effect of fructooligosaccharides on faecal weight in humans has been measured.

Ref.	Number of subjects	Amount oligosacchride	Increase in faecal wet weight (g/g oligosaccharide)	Remarks
Gibson GR et al (45)	8 (7 men, 1 woman)	15 g oligofructose/day, 4 subjects went on with another period with inulin Control sucrose	1.3 for oligofructose 2.0 for inulin	Few subjects, especially on inulin, no statistical treatment of faecal weights
Alles MS et al (46)	24 healthy men 19-28 y	5-15g/day	No significant effect	Negative result possibly due to a high fibre intake (40 g/day) with high stool output (270 g/day)
Castiglia-Delavaud et al (47)	9 healthy young men	50 g/day	1.5 for inulin (the same as for sugar beet fibre)	-

Table 4.Studies on prebiotics and mineral absorbtion, columns 1-14.

1. Ref	2. Main hypothesis	3. Intervention method	4. Study design	5. Recruitment/ Inclusion criteria	6. Exclusion criteria
Coudray et al (90)	Soulble or partly soluble fibre improves absorption and balance of calcium, magnesium, iron and zink	28 day periods, 3x3 latin square design, 40g inulin or 40g sugar beet fibre + 18g fibre from other sources per day	RCT	9 male students	Digestive, hepatic or cardiac disease
Van den Heuvel et al (91)	Inulin, fructooligosaccharides and galactooligosaccharides increase mineral absorption	4x21 days	RCT	12 healthy male subjects 20-30 y Typical Dutch food pattern	Any health problem
Van den Heuvel et al (92)	Moderate dose of oligofructose stimulates calcium absorption in adolescents	2x9 days	RCT, double-blind	12 boys 14-16	

Ref.	7. Matching of groups	8. Treatment time	9. Follow up after treatment (if any, e.g. persistens of probiotic organism in faeces, recurrence of symptoms)	10. Number of subjects/patients		
				Number starting	Number ending experimental period	Number followed up (if any)
90.	Own controls	28 days (12 with maximum fibre intake)	No	9	9	
91.	Own control	21 days Iron absorption measured last 7 days, calcium absorption measured last day	No	12	12	
92.	Own control	2+7 days treatment periods	No	12	12	

Ref	11. Results Main outcome Treatment	12. a. Side effects b. Compliance measure	13. Evaluation of quality	14. Concluding remarks – strength of evidence
90.	Inulin increased apparent Ca absorption by 58%. Both beet fibre and inulin increased absolute absorption. Apparent balance also positive. No effect on Mg, Fe or Zn	No reported	++	Increased calcium apparent absorption by inulin using conventional balance technique. Balance periods short for calcium.
91.	15g inulin, oligofructose or galactooligo-saccharides/day did not alter Ca or Fe absorption (7 days measurement of iron, 24h measurement of Ca)	No reported	++	No effect, probably related to short measurement period for calcium (24h). One ileostomy study (Ellegård et al (38)) support no effect of inulin on the level of small intestine
92.	15 g oligofructose/day increased fractional Ca absorption from 48 to 60% during 36 h	No reported	++	Increased calcium absorption after 36h, allowing for effects on colonic absorption. Isotope technique