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

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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 probiotics 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-induced 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 symbiotic foods. Certain probiotic oligosaccharides may increase calcium absorption. Prebiotics 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 symbiotics 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 vitro 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 probiotics 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, Shirot, Lactobacillus acidophilus, LA1, NFCB 1748, Lactococcus lactis L1A, Bb12, Gaio, Lactobacillus rham-
Health effects of probiotics and prebiotics

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 through 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 sensitivity 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 thermophiles (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 triacylglycerol-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
triaclylglycerol 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 triacylgllycerol 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 Saccharomyces 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**

**Prebiotics**

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. rhamnous* 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. rhamnous* 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 oligosaccharides are completely fermented, their bulking effect would be expected to occur through an increased microbial mass.

Table 3, (page 74) lists three studies (45*,46*) 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 aetiology 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. rhamnusos* 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 therapeutical 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 magnitude as reported for *L. rhamnusos* 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. rhamnusos* 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
Health effects of probiotics and prebiotics

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 should be urgently needed.

Relapsing Clostridium difficile infection

Severe C. difficile infection is treated with antibiotics active against anaerobic bacteria (vancomycin or metronidazole). 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 10¹⁰ 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

Scand J Nutr/Näringsforskning 2001 61
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 neuro-peptides (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 clostridia, 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 *S. salivarius* and *L. rhamnosus* GG showed few relapses (15%) compared to 100% in the control group. This study further supports the potential of probiotics 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 marine knock-out models for IBD suggest that an immunological 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 symbiotic 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 nondigestible 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 lactobacil 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 prerequisite 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 zinc (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 aero-tolerant 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 leukemia patients or in immunosuppression (93). The findings that most clinical LAB isolates belongs to the rhannosus-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 cytosstatic-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 diarrhea are unlikely to occur with a consumption of 20 g fructooligosaccharides (FOS)/day or less for a person weighing 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 pre- and probiotics, 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.

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The article continues with Tables 1-4.
| 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 H₂ 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 lactate contents and bacterial cultures | 10 g of lactuloses and 18 g of lactose in 1. Yougurt 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 mal digesters | 1. 50 g lactose 2. 50 g sucrose 3. 25 g lactose 4. 25 g fructooligosaccharides | 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 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 10⁷ 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 |
| Schaafisma 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 hypercholesterolomic 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 hypertension |
| 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 |
| Agerback 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 | 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) | Fructooligosaccharides reduces serum cholesterol | 1. 8 g per day of fructooligosaccharides 2. 5 g per day of sucrose | RCT no cross-over | Subjects with diabetes |

cont.
| Study Authors | Design | Intervention | Comparator | Subjects |
|---------------|--------|--------------|------------|----------|
| Brighenti et al (30) | RCT | Inulin reduces serum cholesterol | Placebo | Healthy male volunteers |
| Canzi et al (31) | CT | Inulin lowers serum cholesterol | Placebo + Inulin | Healthy subjects |
| Davidson et al (32) | RCT, cross-over | Inulin reduces serum cholesterol | No inulin | Men and women with hypercholesterolemia |
| Pedersen et al (33) | RCT | Inulin lowers serum cholesterol | 14 g of inulin in 40 g margarine | Normolipidemic women |
| Alles et al (34) | CRT | Fructooligosaccharides reduces serum lipids | 15 g fructo-oligosaccharides | Type 2 diabetes |
| van Doccum et al (35) | R double-blind,diet controlled | Oligosaccharides reduces serum lipids | Inulin | Healthy men |
| Luo et al (36) | CRT | Fructooligosaccharides (FOS) influences glucose and lipid metabolism | 20 FOS | Type 2 diabetes |
| Jackson et al (37) | CT | Inulin reduces lipids and glucose | 10 g inulin | Healthy men and women |
| Ellegråd et al (38) | RCT | Inulin lowers serum cholesterol and influences mineral absorption | 17 g inulin | Ileostomy subjects |
| Hata et al (39) | RCT, parallel design | Sour milk reduces blood pressure in hypertensive subjects | 95 ml Calpis, sour milk | Hypertensive subjects |
| Kawase et al (40) | CT | Fermented milk reduces serum lipids and blood pressure | 200 ml fermented milk | |
| Ling et al (41) | CT | Colonization of faeces by Lactobacillus GG and effects on bowel function | 200 ml placebo | |
| Alm (43) | Cross-sectional or cross-over study | Acidophilus milk alleviates constipation | Constipated subjects from a geriatric ward |
| Newcomer et al (49) | Double-blind RCT | Symptoms would be reduced in IBS and lactase-deficient subjects (using acidophilus milk) | IBS (61) | |
| Niedzielin et al (50) | RT, parallel test | Reduction of symptoms | IBS | Inflammatory bowel disease |
| Nobaek et al. (51) | RCT | L. plantarum DSM 9843 reduces abdominal bloating and pain | 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 \( (\text{if any}, \text{e.g. persistence of probiotic organism in faeces, recurrence of symptoms}) \) | 10. Number of subjects/patients |
|------|-----------------------|-------------------|-------------------------------------------------|---------------------------------|
|      |                       |                   |                                                 | Number starting | Number ending experimental period | Number followed up \( (\text{if any}) \) |
| 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 \( (2 \times 39) \) |                                  |
| 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               | 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 \( \text{baseline, test, baseline} \) |                              | 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 \( 1 \)                 |                                  |
| 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. Evaluation of quality | 14. Concluding remarks – strength of evidence |
|------|-------------|---------------------------|------------------------------------------------|
|      | Main outcome | Other effects             | Drop-outs | a. Side effects | b. Compliance measure |
|      | Treatment   | Control                   | Treatment | Control | Treatment | Control |
| 6    | Reduced total H₂ production |                         |           |           |           | +       | Acidophilus milk improves lactose tolerance and digestion |
| 7    | Reduction of symptoms and H₂ production with a yoghurt culture |                         |           |           |           | +       | Less symptoms with yoghurt |
| 8    | Yoghurt delayed breath H₂ 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 H₂ concentrations after the three test products, no difference 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 undigestable carbohydrates |                         |           |           |           | ++ | More symptoms with oligo-saccharides in low lactase activity subjects |
| 14   | S-cholesterol reduced by 0.02 mmol/l | S-cholesterol reduced by 0.07 mmol/l | Unaffected levels of LDL, HDL and triglycerides | 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 | Triglycerides and HDL no change |           |           | ++ | 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 | Triglycerides and HDL no change | Triglycerides 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 |
|   | Studies on lactose intolerance, blood lipids, hypertension and irritable bowel syndrome, columns 11-14 (cont). |
|---|---------------------------------------------------------------------------------------------------------|
| 25 | Lower cholesterol absorption with Verum halsolif | 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% | Total cholesterol down to 159 mg/dL | TG 54.4 ± 4.4 | TG 74.8 ± 7.4 | ++ | Small study TG down |
| 31 | Total cholesterol down to 150 mg/dL | Total cholesterol increased: 7.4% and LDL cholesterol by 12.3% | Triglycerides no difference | ++ | "Reduction" due to an increase in the control group |
| 32 | TC, LDL, HDL no change | TC LDL HDL no change | Triglycerides no change | 6 | Flatulence, cramps | +++ | No effect on serum cholesterol of insulin |
| 34 | No effect on TC, LDL, cholesterol or triglycerides | Glucose metabolism unchanged | ++ | Lack of effect not due to insufficient statistical power |
| 35 | No sign. difference | Inulin reduces triacylglycerol levels (p<0.08) | ++ | Trend for a reduction of plasma TAG |
| 36 | Total cholesterol unchanged | 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 |
| 37 | Insulin reduces triacylglycerol levels (p<0.08) | No sign change | ++ | Blood pressure down, small group, parallel study |
| 38 | Blood pressure down, syst. 14.1 mm Hg, Diast. by 6.9 after 8 wk | HDL increased after 4 weeks. Triglycerides down. Systolic blood pressure was reduced | Bowel frequency increased in 7 of 10 patients to 8 or more /week | ++ | Changes in serum lipids. Lower blood pressure |
| 40 | Colonization of faeces by lactobacilli. No changes in fecal frequency weight or pH | 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 | Reduction in flatulence by 18%, reduction in pain by 18% | 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.
| Ref.       | Main hypothesis                                                                 | Intervention method                                                                 | Study design | Recruitment/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 (2x10⁹-2x10¹⁰ CFU/d)   | RCT          | 4-45 months: Acute diarrhoea of less than 7 days’ duration >3 watery stools last 24 h 82% were positive for rotavirus |
|           |                                                                                 | 2) *L. rhamnosus* GG freeze-dried, same amount                                        |              |                                                                                              |
|           |                                                                                 | 3) Pasteurized yoghurt                                                                |              |                                                                                              |
| 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                | 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) 10⁸-10⁹ CFU/d of *L. reuteri* SD2112                                               | RCT          | 6-36 months of age presenting with acute diarrhoea (1 or more watery stool/d) of less than 7 days’ duration during rotavirus season |
| Sornikova et al (57) | *L. reuteri* shortens duration of rotavirus-induced acute diarrhoea in children | 1) 10⁹, 10¹⁰ CFU (in capsules with lactose)                                          | RCT          | 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*                                          | RCT          | Healthy men and women (5+5), mean age 29 years                                               |
| Orellana et al (59) | Milk fermented with *B. longum* and *L. acidophilus* prevents *C. difficile* colonization and gastrointestinal side effects caused by antibiotic treatment | 1) 250 g fermented milk (*L. bulgaricus* 10⁹ CFU/mL, *S. thermophilus* 10⁹ CFU/mL) with *B. longum* (1-5 x 10⁹ CFU/d) and *L. acidophilus* (5-7 x 10⁶ CFU/d). 15 g oligofructose added 2) Fermented milk with 15 g oligofructose 3) Fermented milk All: 100 mg cephalosporin prepost | RCT          | 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                   | 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 2x10⁹ CFU *L. rhamnosus* GG twice daily                              | 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 metronidazole to prevent *C. difficile* recurrence | 1) 1 g/d of lyophilized *S. boulardii* in capsules (3x10⁶ CFU/d)                      | RCT          | *C. difficile* disease, ranging from uncomplicated diarrhoea to pseudomembranous colitis. Positive *C. difficile* cultures  |
| Bennet et al (65)    | *L. rhamnosus* GG prevents relapse of *C. difficile* diarrhoea                    | 1) 1, 2 or 4 capsules with 2x10⁹ 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. difficile* 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 |
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 | Number followed up |
|      |                       |                   |                             | experimental    | period        | (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. | Until discharge, maximally 5 days | Four weeks after treatment | 1) 19 2) 22 | 1) 19 2) 21 | 7 |
|      |                       |                   |                             |                             |                             | 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 | 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 constience, 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 | 13. Evaluation of quality – strength of evidence | 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 ± 0.5 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 |  |  |  |  | ++ (+) | No data on breast-feeding |
|     |  | 2.5 days | p=0.001 |  |  |  | ++ (+) | Intake of milk product fermented with *L. rhamnosus* GG shortens rotavirus-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). |  |  | ++ | No data on breast-feeding  
Faeces not cultured for any pathogens |
| 57. | Diarrhoea prevalence day 1:  
1) 81% (p<0.01)  
2) 100%  
3) 100%  
Diarrhoea day 2:  
1) 48% (p<0.04)  
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) 10^9 CFU/g  
2) 10^7 CFU/g  
<10^7 CFU/g | ++ (+) | ++ | *L. reuteri* shortens duration of acute 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 |
| 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  
1) 2/10  
3/10  
1 in group 1, due to treatment with other antibiotics | 1 in group 1, due to treatment with other antibiotics |  |  | ++ | Small study |
|     |  |  |  |  |  |  |  | No significant changes in clinical parameters. Possible reduction of *C. difficile* colonization (p=0.08 Fisher’s test) |

**Scand J Nutr/Näringsforskning 2/01**

73
Table 2. Studies on diarrhoea, columns 11-14 (cont).

| Duration of diarrhoea: 2 days | Reduced abdominal distress, pain and flatulence | Blood concentration of erythromycin measured on 1st and last day | (+) Primary data on diarrheal frequency, duration not given in publication |
|------------------------------|--------------------------------------------------|-----------------------------------------------------------------|------------------------------------------------------------------|
| Stomach pain: 25% | 8 days p=0.05 | Stool samples 1st and last days. *L. rhamnosus* GG identified by morphology | Small study |
| 39% p? | | | + Reduced diarrhoea in GG group, but insufficient data given to judge strength of conclusion |

| Diarrhoea (>2 watery stools/day for >1d): 3 (5%) | *C. difficile*-positive individuals: | Faecal cultures were screened for *L. rhamnosus* GG in 23 randomly selected patients. 21/23 had >10^6 carriers. No drop-out analysis |
|-----------------------------------------------|---------------------------------|--------------------------------------------------------------------------|
| No causative agent found in most cases of diarrhoea | 9 (16%) p=0.05 | ++ relatively large study, but large drop-out rate. No drop-out analysis |
| | 1 | | (+++) *L. rhamnosus* GG reduces incidence of antibiotic-associated diarrhoea. Effect not quite significant and large drop-out. |

| *C. difficile* recurrence: 26.3% | 4 deaths (S. aureus sepsis, resp. arrest, cardiac arrest, prostate cancer) | Calculations based on intention-to-treat |
|-----------------------------------------------|---------------------------------|---------------------------------------------------------------------------|
| No of stools/day: 2.1 | 1 death (pneumonia) | Calculations based on intention-to-treat |
| Toxin B in faeces: 6.7% | 30% | a) Increased thirst: p=0.02 |
| No effect on pain, nausea, or cramps. | 44.8% | 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 |

| 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 oligosaccharide | 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 absorption, columns 1-14

| Ref | 2. Main hypothesis | 3. Intervention method | 4. Study design | 5. Recruitment/Inclusion criteria | 6. Exclusion criteria |
|-----|--------------------|------------------------|----------------|----------------------------------|-----------------------|
| Coudray et al (90) | Soluble or partly soluble fibre improves absorption and balance of calcium, magnesium, iron and zinc | 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 | 10. Number of subjects/patients |
|-----|----------------------|------------------|-----------------------------|---------------------------------|
|     |                      |                  | (if any, e.g. persistens of | Number starting | Number ending experimental period | Number followed up (if any) |
|     |                      |                  | probiotic organism in faeces, recurrence of symptoms) |                  |                                  |                              |
| 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          | 2x7 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 galactooligosaccharides/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 |