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review

Pre-, Pro-, Synbiotics and Human Health

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Summary

Western lifestyle is associated with a sustained low grade increase in inflammation leading to an impaired innate immunity and reduced resistance to disease, changes which might explain the epidemic of chronic diseases spreading around the globe. The immune system cannot function properly without access to bacteria and plants, which when raw, are rich not only in bacteria but also in plant fibres, antioxidants, healthy fats and numerous other nutrients. Modern food technology with plant breeding, separation, condensation of food ingredients, heating, freezing, drying, irradiation, microwaving, an effective tool for destroying foods and hereby counteracting optimal immune function, is suspected to be a leading cause of so-called Western diseases. The supply of pre-, pro-, and synbiotics has sometimes proved to be effective tool to counteract, especially acute diseases, but has often failed, especially in chronic diseases. Thousands of factors contribute to unhealthy living. Numerous alterations in lifestyle and food habits are needed, if to prevent and cure 'treatment-resistant' chronic diseases. Among these are avoiding such processed foods that are known to be rich in pro-inflammatory molecules, and also eating substantial amounts of foods with documented anti-inflammatory effects such as turmeric/curcumin, molecules which might be included in future synbiotic compositions.

Key words: prebiotics, probiotics, synbiotics, immune system, resistance to disease

Western Food – A Threat to Human Health

Human life without access to plants and bacteria would be miserable. Plants and bacteria, which have existed for billions of years, often have robust protection systems, which can be used by humans. Our Palaeolithic forefathers did on annual basis receive their daily food from at least five hundred plants and also, as the food they ate was often stored in the soil, a rich supply of various microorganisms. Modern food is based on nutrients received from only a small number of plants; 80 % of the nutrients come from 17 plants and 50 % of the calories from eight grains. Furthermore, the main part of Western foods is extensively processed; treatments like growth enhancement, separation, condensing, drying, freezing, irradiation, burning, microwaving, toasting, adding various ingredients and especially heating are often used. It is well-known that some important plant ingredients start disappearing already when heated above 28 °C, important plant enzymes and microbes above 42 °C, dysfunctioning proteins are formed above 80 °C and heterocyclic amines and also *trans* fatty acids from about 130 °C, and increasing as the heating of the food increases further, all changes having negative effect on human health.

Among the dysfunctioning proteins produced during heating of foods are the so called Maillard products, often referred to as advanced glycation and advanced lipoxidation end products, and abbreviated as AGEs and ALEs, respectively. Among foods rich in AGEs and ALEs are: dairy products, especially powdered milk (frequently used in enteral nutrition and baby formulas, as well as in numerous foods such as ice cream), cheese, bakery products (bread crusts, crisp breads, pretzels, biscotti) and

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cereals (rice crispies), overheated (especially deep-fried and oven-fried) meat and poultry but also fish, drinks like coffee and Coca-Cola, Asian sauces including Chinese soy sause, balsamico products and smoked foods in general – for further information, see Goldberg *et al.* (*1*) and Bengmark (*2*). The consumption of such foods, often main constituents in fast foods, has increased dramatically in recent decades, much in parallel to the endemic of chronic diseases.

Deranged and Dysfunctioning Immune System

Numerous chemical substances, additives to foods and pharmaceutical drugs seem to derange the immune system. It is clear, even if not fully investigated, that a large number of chemicals, when consumed, have a strong negative influence on the immune system and the body's resistance to disease. In the past, priority was not given to the investigation of eventual negative effects of consumed food additives and pharmaceutical drugs on the innate immune systems. It has long been known that antibiotics suppress various immune functions, and especially macrophage activities such as chemiluminescence response, chemotactic motility, bactericidal and cytostatic ability, and similar negative effects have also been seen with other commonly used drugs such as H_2-blockers, proton inhibitors and surface-protection agents.

Several other factors increase the degree of systemic inflammation in the body: impaired hormonal homeostasis increases oxidative stress/release of free radicals, intracellular accumulation of 'waste products', inhibits apoptosis, disturbs repair mechanisms, reduces gene polymorphism, increases premature shortening of telomeres and reduces immune defence and resistance to disease, changes often observed in premature aging and in various several chronic diseases (*3*); low level of vitamin D in the body and subsequent secondary hyperparathyroidism (*4*); low levels of antioxidants in the body such as folic acid and glutathione and increased levels of homocysteine (*5*); high levels of estrogens in the body, especially 17β-estradiol, often induced by high consumption of hormone-rich dairy products (*6*); high levels of angiotensin/rennin (*7*); and larger intake of glutenoids (*8*).

The reason why attempts to reduce inflammation with the use of probiotics sometimes failed in the past might be that the pro-inflammatory pressure is simply too high due to the underlying disease, but also due to the consumption of too much of pro-inflammatory foods and prescription drugs, all with inflammation-enhancing abilities. It is likely that under certain conditions, additional measures are needed in order to achieve successful treatment with probiotics. Measures such as reduced supply of pro-inflammatory foods, restriction in the use of pharmaceuticals and increased intake of plant foods rich in anti-inflammatory vitamins and antioxidants, especially various polyphenols, might well be needed – see further below.

Effects of Plant Fibres on Systemic Inflammation

It should be observed that various seeds, nuts, beans and peas are especially rich in fibre, foods which no longer are eaten in the quantities they deserve. A common recommendation of minimum daily fibre intake is in the range of 30–35 g per day, which roughly corresponds to about half a kg of fruits and vegetables, or, as often expressed, 5 to 8 fresh fruits and vegetables per day. The recommendations for children above the age of 2 are usually defined as age+5 g per day. No precise recommendation exists yet about the intake of fibre under different conditions of disease. The daily intake of dietary fibre is unsatisfactory in all Western countries, especially among people with low level of education and low income. In the US for example, the estimated daily intake of fibre is approx. 14–15 g per day or about 50 % of what is recommended, and far below the 60–80 g per day of substrate required to maintain a large bowel flora of 10^{14} microorganisms, known to be typical for a healthy and well-functioning human colon. Most Americans and Europeans have lost the ability to maintain a large proportion of what can be regarded as a natural flora (*9*). A recent study in a North-European population has found *Lactobacillus plantarum, Lactobacillus rhamnosus* and *Lactobacillus paracasei* ssp. *paracasei* on the rectal mucosa of healthy humans only in 52, 26 and 17 %, respectively (*10*). The colonization rate with other, commonly milk-born probiotic bacteria, such as *Lactobacillus casei, Lactobacillus reuteri* and *Lactobacillus acidophilus* was in the same study only 2, 2 and 0 %, respectively.

Commonly consumed cooked roots and other starchy vegetables or grains consumed as bread, cereals and porridge, but also most of the fruits consumed in Western countries contain relatively little fibre, usually no more than 1–3 g per serving. The largest amount of consumed plant fibre is provided by resistant starch (raw potato, unripe green banana), which re-crystallize when allowed to cool after cooking, especially important for potato and bread. The daily consumption of this type of fibre varies from one individual to another with several hundred per cent (approx. 8–40 g/day). The second largest source of fibre is non-starch polysaccharides (approx. 8–18 g/day). The third group of fibre is oligosaccharides (onions, artichoke, banana, chicory), which although important for health, regrettably today are consumed in much too small quantities (approx. 2–8 g/day) (*11*).

Function of Dietary Fibres

Supplemented fibres are associated with several health benefits. The best documented physiological effects, in addition to providing energy and nutrients to the host and flora, are that they:

- change mucosal structure, increase mucosal growth and improve mucosal function;
- increase intestinal flora, relieve constipation, reduce production of putrefactive gases and provide resistance to invading microorganisms;
- reduce serum triglycerides, serum cholesterol and VLD lipoproteins;
- reduce the glycemic response to eating;
- improve water and electrolyte balance and increase bioavailability and absorption of minerals such as Ca, Mg, Fe and Zn.

Consumption of medical fibres should always be regarded as a surrogate for not consuming enough fresh fruits and vegetables. There is no solid information to support that supplementation of medical fibres to healthy individuals eating a diet rich in fruits and vegetables is associated with additional health benefits. Medical fibres are mainly needed because the individual has lost the ability to consume enough fresh fruits and vegetables. This is often the situation in persons with severe allergy, in old and debilitated persons and in persons with some GI disorders, such as short bowel syndrome and advanced diverticular disease. This is also most often the condition of critically ill patients, for whom enteral supply of concentrates of medical fibres has become a most valuable clinical tool. It must, however, always be remembered that during processing bioactive fibres lose numerous important antioxidants and nutrients, some of which when possible should be separately supplemented, and whenever possible complemented with the supply of fresh fruits and vegetables.

Health Benefits of Increased Plant Fibre Consumption

Significant information on beneficial effects from increased intake of plant fibres and so-called prebiotics exists mainly for two large groups of diseases, described in the following chapters.

Blood glucose control/prevention of type 2 diabetes

Fibre is a slow release system for delivery of glucose to the body. Fibre, regularly supplied to patients with diabetes, will significantly reduce the level of blood glucose and the need of insulin. Studies suggest that the most pronounced effects of fibres on glycemic index are obtained by water-soluble fibres. Guar gum is in this respect by far the most clinically tried fibre and will, as based on 15 different studies, reduce blood glucose to almost half (44 %).

Lipid control/prevention of coronary heart disease

Soluble fibres such as pectin, guar gum, β-glucans (oat) reduce significantly blood cholesterol both in hypercholesterolemic and normocholesterolemic individuals, effects not found when non-soluble fibres such as cellulose and wheat bran are tried. Soluble fibres are excellent substrates for the production of short chain fatty acids (SCFAs) in the large intestine, known to reduce the levels of cholesterol(s) in the body.

A meta-analysis reports statistically significant protective effects against coronary heart disease in 14 out of 16 studies (*12*). In addition, fibre consumption will also reduce clotting and increase fibrinolysis, also important for prevention of building of arterial wall plaques and prevention of thrombosis formation.

Clinical Use of Fibres

Substances important for health: amino acids such as arginine, glutamine, histidine, taurine, various sulphur and related amino acids, polyamines, ω-fatty acids, numerous vitamins and antioxidants are all to a great extent supplied to the body from plants. One cannot expect any significant amount of antioxidants to be delivered to the lower level of the gastrointestinal tract if not 'hidden' in plant fibres. It is important to remember that key nutrients such as ω-3 fatty acids, glutamine, glutathione and several other nutrients are heat-sensitive and do not tolerate processing or storage to any larger extent. Plant fibres, which have been dried, heated up or microwaved cannot be expected to contain any larger amounts of these; they do mainly come with unprocessed foods. It is highly desirable that, whenever possible, the supply of commercial nutrition formulas is complemented with the supply of fresh fruit and vegetable juices, as locally produced as ever possible. It is also desirable that several fibres are supplied in parallel, and that both soluble and non-soluble fibres are used. For example, oat fibres are mainly metabolized in the proximal colon, while wheat fibres are known to be effective in the distal part of the colon, *e.g.* the part of the colon where most cancers are localized. Oat has mainly shown sepsis-reducing effects, while wheat has mainly been effective in cancer prevention.

Among the fibres commonly used in clinical nutrition are:

Algal fibres

Most of the algal fibres are resistant to hydrolysis by human endogenous digestive enzymes, but are to various degrees fermented by colonic flora. The soluble fibres consist of lamarans (a sort of β-glucan associated with mannitol residues), fucans (sulphated polymers associated with xylose, galactose and glucuronic acid) and alginates (mannuronic and guluronic acid polymers). The insoluble algal polymers consist mainly of cellulose. Fermentation of alginates yields high levels of acetate (80 %), while lamarans yield preferably butyrate (16 %). It is most likely that algal fibres will within a few years be routinely used in clinical nutrition.

Fructans

Fructans, starches and sucrose serve in the plant as its energy reserve. These substances are also produced by bacteria and fungi. Fructans are said to enhance the tolerance of the plant to stressful conditions and make it possible for the plants to survive under harsh conditions, such as low temperature and draft. The most well-known fructans are inulin (rich in chicory, artichoke, onions, and banana) and phleins (rich in various grasses). So far inulin has mainly been used in human nutrition. Various oligosaccharides are reported to stimulate the flora and especially the growth of *Lactobacillus* and *Bifidobacterium* in the large intestine and to reduce the content of potentially pathogenic microorganisms (PPMs) in the intestine. Increase in the *Bifidobacterium* flora is regarded as especially favourable since bifidobacteria are known to produce important vitamins, among them thiamine, folic acid, nicotinic acid, pyridoxine and vitamin B_{12}, which is of great importance for health. A fructan called neokestose, found in onion, is reported to have even better ability than inulin to promote the growth of lactic acid bacteria (LAB). Also, supplementation of fructans is reported to reduce concentrations in serum of

insulin, cholesterol and triacylglycerol. It is also reported to promote the absorption of calcium and other minerals. Other oligosaccharides such as those extracted from peas and beans, especially soya bean oligosaccharide (raffinose and stachyose) and pyrodextrin, produced by pyrolysis of maize and potato starch, are also reported to be beneficial for human health.

Glycomannans

Glycomannan is a glucose/mannose polymer derived from a plant called *Amorphophallus konjak*, which has several English names such as devil's tongue, elephant yam and umbrella arum. It has unique hydroscopic abilities and in contact with water it swells and forms a viscous gel, which, like other gels, delays gastric emptying and intestinal transit time. It has been shown to be effective in delaying absorption of digestible energy. It has this far mainly been used in Japan and other Asian countries to treat diabetes, hypertension and hypercholesterolemia. Dietary supply of konjak mannans has been shown to alter the flora and reduce tumorigenesis in experimental animals. It is also effective in the control of diarrhoea in enteral nutrition, especially in elderly patients, and in the increase of the *Bifidobacterium* flora.

Oat gum

Oat contains a series of interesting compounds, which is the reason why an increasing part of the world production of oat goes to the pharmaceutical and cosmetic industries. The amino acid pattern of oat is rather similar to that of human muscle (only that of buckwheat is more alike), and can thus be expected to deliver most of the amino acids needed to build muscles. Oat is rich in water-soluble fibres, β-glucans, and is known for its antiseptic properties. It is also rich in natural antioxidants, particularly ferulic acid, caffeic acid, hydrocinnamic acid, and tocopherols. Also, before synthetic antioxidants were available, oat was extensively used to preserve foods: milk, milk powder, butter, ice cream, fish, bacon, sausages and other food products sensitive to fat oxidation. Another ingredient richly available in oat is inositol hexaphosphate (phytic acid), a strong antioxidant, particularly known to enhance natural killer cell activity and to suppress tumour growth. Oat is also rich in polyunsaturated fats/polar lipids such as phosphatidylcholine, known for their protective effects of mucosal and cellular surfaces.

Pectin

Pectin is also an interesting fibre, extensively used by pharmaceutical and food industry. It has a unique ability to form gels and is commonly used as a carrier of pharmacologically active substances and in baby foods. An important finding is that pectin is a very strong antioxidant against the three most dominant oxidation damages induced by peroxyl, superoxide and hydroxyl radicals. These effects might explain why pectin has the capacity to stimulate the gut-associated immune system and to prevent disruption of the intestinal microflora. Pectins have shown strong protective and healing effects on gastric but also intestinal mucosa in experimental studies not inferior to what is observed with H_2-blockers, proton inhibitors and surface-protection agents (*13, 14*). Pectin builds a protection layer in the stomach and facilitates maintenance of gastric acidity, important for prevention of colonization of the stomach by pathogens. Pectin is also an excellent substrate for microbial fermentation.

Lactic Acid Bacteria as a Key to the Fermentation of Fibres

Not all fibres are easily fermented in the gut. Among the more fermentation-resistant fibres are wheat fibres, which are usually not digested until they reach descending colon. Also, oligofructans (inulin or phleins) are difficult to ferment and only a small minority of LAB are able to do so. When the ability of 712 different LAB to ferment oligofructans was studied, only 16 were able to ferment the phleins and 8 to ferment inulin (*15*). Apart from *Lactobacillus plantarum*, only three other LAB species, *Lactobacillus paracasei* ssp. *paracasei*, *Lactobacillus brevis* and *Pediococcus pentosaceus*, were able to ferment these semi-resistant fibres. Another study investigated the ability of 28 different LAB to ferment pure fructooligosacharides (FOS). All *L. plantarum*, *L. casei* and *L. acidophilus* strains studied and most bifidobacteria metabolized FOS, in contrast to yoghurt bacteria such as *L. bulgaricus* and *Streptococcus thermophilus* and also *Lactobacillus strain* GG, which were all unable to ferment these fibres (*16*).

Clinical Experience with Supplemented Plant Fibres

Plant fibres in constipation

Chronic constipation is one of the most common disorders in Western countries. Its aetiology remains unclear despite numerous clinical, pathophysiologic, and epidemiologic studies, but it is suggested that high intake of dairy products and intake of plant fibres play a significant role in its pathogenesis. A randomized sample of 291 children with idiopathic chronic constipation was compared in a case control study with 1602 healthy controls (*17*). Constipation was clearly negatively correlated with low intake of cellulose and pentose fibres ($p<0.001$). Fructooligosaccharides (FOS) may also have potential benefits in constipation, since they exhibit many soluble dietary fibre-like properties. In a study a total of fifty-six healthy infants, aged 16–46 weeks (mean age 32 weeks) were randomly assigned to receive either 0.75 g of FOS or placebo added to a serving of cereals for 28 days (*18*). The mean number of stools per infant was 1.99±0.62 per day in the FOS-supplemented group compared with 1.58±0.66 in the control group ($p=0.02$).

Plant fibre to prevent and treat diarrhoea

In a large randomized study in acutely ill medical and surgical patients, all requiring enteral nutrition for a minimum of 5 days, supplementation of hydrolyzed guar gum was compared to a fibre-free enteral nutrition, the incidence of diarrhoea being 9 % with fibre-supplementation, compared to 32 % with fibre-free nutrition ($p>0.05$) (*19*). One of the effects of certain fibres is that they in-

crease the bioavailability and absorption of zinc, which is especially shown for oligosaccharides. Zink supplementation was in a randomized study in 3- to 59-month-old children in Bangladesh proven effective in lowering both the incidence of diarrhoea and its duration (*20*). In another study from Bangladesh, 250 g/L of green (unripe) banana (equivalent to two fruits) or 2 g pectin/kg food were supplemented to a rice diet in children suffering from persistent diarrhoea (*21*). The amounts and frequency of stools, the duration of diarrhoea, numbers of vomiting, use of oral rehydration and amounts of given fluid solutions were all significantly reduced in both cases, supplementation with green banana and pure pectin. Recovery on the third day was seen in 59 % in the green banana group, in 55 % in the pectin group, compared to 15 % in the rice only control group.

Plant fibre to support mineral absorption

It is well accepted that nutrition is of great importance for bone health. Most of the interest has this far focused on calcium and vitamin D. Much less interest has been paid to other important nutrients such as protein, and especially to minerals such as phosphorus, potassium, magnesium and vitamins such as C and K. Recent studies suggest that increased intake of plant fibres, fruits and vegetables is associated with an increased bone mineral density also in elderly subjects, both women and men (*22,23*). Of pure fibres available, mainly the effects of oligosaccharides have been studied, and mainly in experimental animals, while calcium absorption, bone calcium content, bone mineral density, bone balance and bone formation/bone absorption index have been reported to significantly increase already after three weeks of supplementation of a mixture of inulin and fructo-oligosaccharides.

Plant fibre to control mass

No major effects on body mass by supplementation of only prebiotic fibre have this far been reported. The effects of dietary fibre on subjective hunger ratings and weight losses were studied some twenty years ago in members of a weight loss club. One hundred and eight of 135 members completed the trial: 23 controls, 45 on ispaghula granulate and 40 on bran sachets (*24*). Both fibre preparations reduced hunger at all meals. The mean±S.D. mass reductions during the trial were (4.6±2.7) kg for the controls, (4.2±3.2) kg for the ispaghula group and (4.6±2.3) kg for the bran group ($p>0.05$ for both groups). Although supply of dietary fibre immediately before meals did reduce the feeling of hunger, it did not provide any additional benefits to the weight reduction. A more recent crossover study compared the effect on satiety of supplementation of (27±0.6) g/day of fermentable fibres (pectin, β-glucan) with similar amounts of non-fermentable fibre (methylcellulose). The daily satiety was increased more significantly with non-fermentable (methylcellulose) than with fermentable fibres (β-glucan, pectin) ($p=0.01$), but no differences were observed in daily energy intake or loss of body mass or body fat (*25*).

Plant fibre in inflammatory bowel diseases (IBD)

Although both patients with IBD and irritable bowel syndrome (IBS) are known to under-consume dietary fibres, there is little evidence that the lack of dietary fibre plays a role in the pathogenesis of these diseases. The ability of maintaining remission in ulcerative colitis (UC) patients by a daily supply of 10 g of *Plantago ovata* seeds (also called psyllium or ispaghula husk) was compared with daily treatment with 500 mg of mesalamine and a combination of the two (*26*). Twelve months of treatment failed to demonstrate any difference in clinical benefits between the three groups. Germinated barley foodstuff (GBF), a by-product from breweries, rich in hemicellulose and in glutamine, was tried in 39 patients with mild-to-moderate active UC (*27*). Daily supply of 30 g reduced significantly the disease activity, increased the concentration of short-chain fatty acids (SCFAs), and increased the numbers of *Bifidobacterium* and *Eubacterium* in the stool. It can well be that the observed effect was more due to increased supply of glutamine and other antioxidants such as various B vitamins than to the fibre *per se* as these compounds are known to be rich in by-products from breweries. Glutamine, as well as other antioxidants, is known to attenuate pro-inflammatory cytokines such as TNF-α and to enhance the release of heat shock proteins (HSP-72) (*28*). A controlled study using oat bran as fibre source was recently reported from a study in 22 patients+10 controls with quiescent UC. Daily supply during three months of as much as 60 g of oat bran (equivalent to 20 g of dietary fibres) resulted in a significant increase in faecal butyrate (36 % average) but also in reduction in abdominal pain. All the treated patients tolerated well the large dose of fibre and signs of relapse of disease were seen in none of the colitis patients (*29*). Butyrate has been shown to inhibit NF-κB activation of lamina propria macrophages, and to reduce the number of neutrophils in crypts and surface epithelia, as well as the density of lamina propria lymphocytes/ plasma cells in patients with ulcerative colitis (*30*) – findings correlating well with the observed decreased disease activity. Twenty patients with ileal pouch-anal anastomosis received 24 g of inulin daily for two weeks. Significant reduction in inflammation was observed with endoscopy and histology. In addition, significant increase in faecal butyrate concentrations and reductions in faecal pH, faecal content of secondary bile acids, and growth of *Bacteroides fragilis* were observed (*31*).

Plant fibre in irritable bowel disease

Dysmotility disorders are increasingly common in Western societies. Some evidence suggests that various dysmotility disorders, gastroesophageal reflux problems, infant colic and constipation are all food-related features, and often due to intolerance to cow's milk proteins (*32*). Irritable bowel syndrome (IBS) is a clinical diagnosis based on the occurrence of abdominal distension, abdominal cramps, often increased transit time, more frequent stools, and relief of pain on defecation. The prevalence of the syndrome varies between 7 and 22 %, making IBS the most common functional gastrointestinal disorder (*33*). Unfortunately, no effective pharmaceutical treatment exists or if existing is unacceptably toxic (*34*). This

has resulted in a need for additional modalities for the treatment of IBS. Pre- and probiotics appear in this perspective as attractive alternatives, see further reviews (*35, 36*), especially as early data from human intervention studies and especially results from recent animal studies clearly indicate that prebiotics have an impact on the immune system: immune cells of the gut-associated lymphoid tissue (GALT) including Peyer's patches are primarily responsive to the oral administration of prebiotics (*37*). However, a consequence of feeding the currently favoured prebiotics (inulin, fructooligosaccharides (FOS), *trans*-galactooligosaccharides and lactulose) is increased gas production in the gut, which might preclude prebiotic use in diarrhoea-predominant IBS, or where bloating or gas are prominent symptoms, but might allow their mild laxative properties to be useful in constipation--predominant IBS (*38*). During the years some small open trials have been performed, but this far, no larger and randomized trial has been reported. However, a recent small open label trial supplementing 15 g/day of a mixture of oligofructose (70 %) and inulin (30 %) reports significant reduction in disease activity (Harvey-Bradshaw index fell from 9.8, S.D.=3.1, to 6.9, S.D.=3.4, $p<0.01$) in parallel to a significant increase in faecal bifidobacteria concentration (from 8.8, S.D.=0.9, log to 9.4, S.D.= 0.9, log cells/g dry feces, $p<0.001$). Also the IL-10 positive dendritic cells increased (from 30 to 53 %, $p=0.06$), and the percentage of dendritic cells expressing TLR2 and TLR4 increased from 1.7 to 36.8 %, $p=0.08$, and from 3.6 to 75.4 %, $p<0.001$), respectively (*38*), which offers hope for the future.

Plant fibre in abdominal pain

Other dietary fibres have also been tried in various groups of abdominal pain. A recent Cochrane review was unable to find any evidence that fibre supplements, lactose-free diets or lactobacillus supplementation are effective in the management of children with recurrent abdominal pain (*39*). However, a study in adult patients reports significant success with other fibres than the classical prebiotics. One hundred and eighty-eight adult IBS patients were classified as having diarrhoea-predominant, constipation-predominant, or changeable bowel habits and were randomly assigned to groups receiving 30 g/day of wheat bran or 5 g/day of partially hydrolyzed guar gum (PHGG) (*40*). After four weeks, patients were allowed to switch group, depending on their subjective evaluation of their symptoms. Both fibres and PHGG were effective in improving pain and bowel habits. Significantly more patients switched from fibre to PHGG (49.9 %) than from PHGG to fibre (10.9 %) at four weeks. Intention-to-treat analysis showed a significantly greater success in the PHGG group (60 %) than in the fibre group (40 %). In addition, significantly more patients in the PHGG group reported a greater subjective improvement than those in the fibre group. It was concluded that improvements in core IBS symptoms were observed with both bran and PHGG, but the latter was better tolerated and preferred by patients (*40*).

Plant fibre to control infections

In an effort to prevent nosocomial pneumonia and sepsis, patients with severe multiple trauma were treated with β-1,3-polyglucose (glucan) – a component of cell walls of plants and microbes (*41*). Pneumonia occurred in 2 out of 21 glucan-treated and in 11 out of 20 patients in the control group ($p<0.01$). Infectious complications (pneumonia and/or general sepsis) occured in 14 % of glucan-supplemented patients *vs.* 65 % in the control group ($p<0.001$). Another study compared the effects of a high-protein formula enriched with fibre but also arginine, and antioxidants with a standard high-protein formula in early enteral nutrition in critically ill patients (*42*). The supplemented group had, in comparison with non-supplemented controls, a lower incidence of catheter-related sepsis (0.4 episodes/1000 ICU days) than the control group (5.5 episodes/1000 intensive care unit (ICU) days) ($p<0.001$), but no differences in the incidence of ventilator-associated pneumonia (VAP), surgical infection, bacteremia, urinary tract infections, mortality and in long--term survival were observed between the groups (*42*).

Pre-, Pro- and Synbiotics Improve Innate Immunity

Several plant fibres (prebiotics) and a few LAB (probiotics) have documented significant effects to improve the function of the innate immune system, the physical barrier, and increase resistance to disease. The hope is that combined supply of these components shall have synergistic rather than additive effects in boosting the immune system and enforcing the barrier functions. Products which combine pre- and probiotics are called synbiotics and treatments using the combination are called synbiotic treatments.

Choice Strains for Probiotic Use

The choice of pre- and probiotics must be based on scientific evidence. Stronger bioactivities cannot be expected from LAB such as yoghurt bacteria, chosen mainly for their palatability. The strains to use must be done with care and extensive preclinical studies are necessary prerequisites.

It is important to remember that the majority of LAB have much limited or no effects on the immune functions and outcome. Constructing synbiotic formulations is especially demanding as most of the LAB used by industry have limited or no ability to ferment bioactive fibres such as inulin or phlein (*15*), no ability to adhere to human mucus, have low antioxidant capacity and, most importantly, do not survive the acidity of stomach and bile acid content.

It has been observed that strains that carry the same name have different and sometimes opposing functions. A recent study has investigated the ability of 46 different *Lactococcus lactis* strains to induce the production of the cytokines interleukin (IL)-6, IL-12 and tumor necrosis factor (TNF)-α. The extent of induction of IL-6, IL-12 and TNF-α was shown to be strain-specific and was not related to species, bio-variety, or the source of the isolate. The production of IL-6 varied between 138 and 0 ng/mL, of IL-12 between 3 and 0 ng/mL and of TNF-α between 20 and 0 ng/mL (*43*).

Unfortunately, few studies have looked at the synergistic effects of simultaneous supply of LAB and fibres – synbiotics. Natural foods supply both LAB and a great variety of plant fibres. Combination of several fibres has been shown to lead to additive effects on microbial ecosystem and immune responses (*44*), and multi-species probiotics are documented to be superior to single-species probiotics, showing increased ability to enhance growth, reduce antibiotic-associated diarrhoea, to prevent infections (*S. typhimurium*) and reduce pathogenic colonisation (*E. coli*) (*45*). Although some studies have used various synbiotic compositions, only two such compositions have been produced after extensive preclinical studies:

(*i*) A unistrain/unifibre composition (Probi AB, Lund, Sweden), produced by fermentation of oatmeal with *L. plantarum* strain 299, containing 10^9 of LAB and approx. 10 g of oat fibre (*46*). In a few studies a commercial fruit juice ProViva™ containing 10^7 of a related *L. plantarum* strain called 299V (Skånemejerier, Malmö, Sweden) is also tried.

(*ii*) A multistrain/multifibre composition, called Synbiotic 2000™, consisting in a mixture of 10^{10}, and a Synbiotic Forte™ with 10^{11} of each of four LAB: *Pediococcus pentosaceus* 5–33:3, *Leuconostoc mesenteroides* 32–77:1, *Lactobacillus paracasei* ssp. *paracasei* 19, and *Lactobacillus plantarum* 2362, and 2.5 g of each of the four fermentable fibres (prebiotics): β-glucan, inulin, pectin and resistant starch (Synbiotic AB, Höganäs, Sweden) (*47,48*).

Lund University microbiologists Åsa Ljungh and Torkel Wadström developed this multi-strain/multi-fibre synbiotic formula, which in recent years has been extensively used in clinical trials. The choice of LAB for the formulation was done after extensive studies of more than 350 human (*47*) and more than 180 plant microbial strains (*48*) and based especially on the ability of the LAB to produce bioactive proteins, transcribe NF-κB, produce pro- and anti-inflammatory cytokines, produce antioxidants, and most importantly, to functionally complement each other. In recent studies, both Synbiotic 2000 Forte™ and Probiotic 2000 Forte™ (no fibre added), containing 10^{11} of each of the four LAB, *e.g.* 400 billion LAB per dose, have been tried.

Plantarum, Paracasei and *Pediococcus*

As mentioned above, when the ability of 712 different LAB to ferment oligofructans was studied, only 16 were able to ferment semi-resistant fibres, phleins, and 8 fermented inulin; identified as *Lactobacillus plantarum, Lactobacillus paracasei* ssp. *paracasei*, *Pediococcus pentosaceus* and *Lactobacillus brevis* (*15*). Interesting clinical results are also often obtained when these LAB are involved. When more than 100 LAB were compared, *L. paracasei* ssp. *paracasei* were demonstrated to be the strongest inducer of Th1 and repressor of Th2 cytokines (*49*). Several other studies have also documented the unique ability of *L. paracasei* to induce cellular immunity, stimulate production of suppressive cytokines as TGF-β and Il-10, suppress CD4 T-cells, Th2 activity, splenocyte proliferation and decrease antigen-specific IgE and IgG1 (*50–53*).

The effect of *Lactobacillus paracasei* (NCC 2461), *Lactobacillus johnsonii* (NCC 533) and *Bifidobacterium lactis* Bb12 (NCC 362) on the induction and maintenance of oral tolerance to bovine β-lactoglobulin (BLG) was investigated in mono-colonized germfree mice. The effects of *L. paracasei* were reported superior to those of the other two (*53*). A study which compared the ability of 50 different LAB to control 23 different pathogenic *Clostridium difficile* found more than half (27 out of 50) totally ineffective, 18 antagonistic to some, but only five strains effective against all: two strains of *L. paracasei* ssp. *paracasei* and three strains of *L. plantarum* (*54*). Another study compared the effects of either *Lactobacillus paracasei*, *Lactobacillus johnsonii*, *Bifidobacterium longum*, or *Bifidobacterium lactis* in rats during 10 to 21 days after *Trichinella spiralis*-induced infection; *L. paracasei* but not the other LAB attenuated muscle hypercontractility, reduced the infection-associated Th-2 response and muscle levels of TGF-β, COX-2 and PGE2 (*55*). A recent study on animals compared the effects of three probiotic strains: *Bifidobacterium lactis* NCC362, *Lactobacillus johnsonii* NCC533 and *Lactobacillus paracasei* NCC2461 on stress-induced changes in gut permeability and on sensitivity to colorectal distension (CRD). Only *L. paracasei* reduced significantly the existing visceral pain and also restored normal gut permeability (*56*).

Synbiotic 2000 in Clinical Medicine

During the last two decades I have studied the effects of synbiotic compositions in various clinical situations, during the 1990s the monostrain/monofibre composition, mentioned above, and in the 2000s the multistrain/multifibre composition, also mentioned above; Synbiotic 2000 and 2000 Forte. Here follows a summary of the effects observed this far. It has been tried under the following conditions:

Acute pancreatitis

Sixty-two patients with severe acute pancreatitis (SAP) (Apache II scores: Synbiotic 2000-treated 11.7±1.9, controls 10.4±1.5) were given either two sachets per day of Synbiotic 2000™ (2×40 billion LAB per day and totally 20 g of fibres) or the same mass of fibres (20 g) as in Synbiotic 2000™ during the first 14 days after arrival to hospital (*57*). Out of 33 patients in the Synbiotic 2000--treated group and 29 patients in the fibre-treated group, nine (27 %) and 15 (52 %) developed subsequent infections respectively, while eight (24 %) of the Synbiotic 2000-treated and 14 (48 %) of the fibre-treated patients developed systemic inflammatory response syndrome (SIRS), multiple organ failure (MOF) or both ($p<0.005$). A total of seven pathogenic microorganisms were cultivated in the Synbiotic-treated group compared to seventeen in the fibre-treated group (Table 1).

Polytrauma

Two prospective randomized trials, one with Synbiotic 2000 and one with Synbiotic 2000 Forte have been concluded. The first study in patients with acute extensive trauma compared Synbiotic 2000 (40 billion LAB/day) with soluble fibre, a peptide diet (Nutricomp, Braun Inc, Germany) with supplementation of glutamine treatments. Treatment with Synbiotic 2000™ led to a highly significant decrease in the number of chest infections (4

Table 1. Isolated bacteria in acute pancreatitis treated with Synbiotic 2000 or fibres

Isolated bacteria	Synbiotic-treated	Fibre-treated
Enterococcus faecalis	1	11
Escherichia coli	0	3
Enterobacter cloacae	0	2
Pseudomonas aeruginosa	0	2
Staphylococcus aureus	0	1
Total	1	18

out of 26 patients, 15 %), compared to peptide diet (11 out of 26 patients, 42 %, p<0.04), glutamine (11 out of 32 patients, 34 %, p<0.03) and fibres (12 out of 29 patients, 41 %, p<0.002) (*58*). Also, the total number of infections was significantly decreased: 5 out of 26 patients (19 %) treated with Synbiotic 2000™, 17 out of 29 patients (59 %) with fibres, 13 out of 26 patients (50 %) with peptide and 16 out of 32 patients (50 %) with glutamine.

In the second study, sixty-five polytrauma patients were randomized to receive once daily for 15 days Synbiotic 2000 Forte (400 billion LAB+10 g of fibres, see above) or maltodextrine as placebo. Significant reductions were observed in the number of deaths (5/35 *vs.* 9/30, p<0.02), severe sepsis (5/35 *vs.* 13/30, p<0.02), chest infections (19/35 *vs.* 24/30, p<0.03), central line infections (13/32 *vs.* 20/30, p<0.02), and ventilation days (average 15 *vs.* 26 days) (*59*). A total of 54 pathogenic microorganisms were cultivated in the Synbiotic-treated group compared to 103 in the placebo group. The time of progression of primary bacteraemia was longer among patients treated with Synbiotic 2000 Forte compared with placebo (p=0.0237 between groups). Twelve (33.3 %) and five (13.9 %) placebo- and synbiotic-treated patients, respectively, developed ventilator-associated pneumonia with *Acinetobacter baumannii* as a bacterial cause (p=0.047 between groups). Treatment with Synbiotic 2000 Forte was accompanied by reduction of white blood cell counts and lipopolysaccharide (LPS) and C-reactive protein (CRP) levels in patients who either did or did not develop sepsis (*60*).

Abdominal surgery

In a randomized controlled study, forty-five patients undergoing major surgery for abdominal cancer were divided into three treatment groups: (*i*) enteral nutrition (EN)+Synbiotic 2000 (LEN), (*ii*) EN+only the fibres of the same mass (20 g) as in Synbiotic 2000™ (FEN), and (*iii*) a standard parenteral nutrition (PN). All treatments lasted for 2 preoperative and 7 postoperative days. The incidence of postoperative bacterial infections was 47 % with PN, 20 % with FEN and 6.7 % with LEN (p<0.05) (personal information). A total of 34 pathogenic microorganisms were cultivated in the Synbiotic-treated group compared to 54 in the fibre-only group. Significant improvements were also documented in prealbumin (LEN, FEN), C-reactive protein (LEN, FEN), serum cholesterol (LEN, FEN), white cell blood count (LEN), serum endotoxin (LEN, FEN) and IgA (LEN).

In another prospective randomized double-blind trial performed on 80 patients subjected to pylorus-preserving pancreatoduodenectomy (PPPD), they received twice daily either Synbiotic 2000™ (2×40 billion LAB) or only fibres from the day before surgery and during the first seven postoperative days (*61*). A highly significant difference in infection rate (p=0.005) was observed as only 5 out of 40 patients (12.5 %) in the Synbiotic 2000-treated group suffered infections (4 wound and one urinary tract infection) *vs.* 16 out of 40 (40 %) in the fibre-treated group (6 wound infections, 5 peritonitis, 4 chest infections, 2 sepsis, and one of each of urinary tract infection, cholangitis and empyema). The infecting microorganisms in the Synbiotic-treated group were: *Klebsiella pneumoniae* (2 patients), *Enterobacter cloacae* (2 patients), *Proteus mirabilis* (1 patient) and *Enterococcus faecalis/faecium* (1 patient) and in the fibre-treated group *Enterobacter cloacae* (8 patients), *Enterococcus faecalis/faecium* (7 patient), *Escherichia coli* (7 patient), *Klebsiella pneumoniae* (2 patients), *Staphylococcus aureus* (2 patients), and *Proteus mirabilis* (1 patient), Table 2. Statistically significant differences between the groups were also observed in the use of antibiotics (mean: Synbiotic 2000 (2±5) days, fibre-treated (10±14) days).

Table 2. Isolated bacteria in patients undergoing pancreatoduodenectomy and treated with Synbiotic 2000 or fibres

Isolated bacteria	Synbiotic-treated	Fibre-treated
Enterobacter cloacae	2	8
Enterococcus faecalis/faecium	1	7
Escherichia coli	0	7
Klebsiella pneumoniae	2	2
Proteus mirabilis	1	1
Staphylococcus aureus	0	2
Total	6	27

Chronic liver disease and liver transplantation

Fifty-eight patients with liver cirrhosis suffering from the so-called minimal encephalopathy were randomized into three treatment groups: group 1 (20 patients) received Synbiotic 2000 (40 billion LAB), group 2 (20 patients) received the same amount of fibres in Synbiotic 2000 and group 3 (15 patients) received placebo (non-fermentable, non-absorbable fibre – crystalline cellulose) (*62*). A significant increase in intestinal LAB flora was observed after one month of supplementation in the Synbiotic-treated group, but not in the other two groups. Intestinal pH was significantly reduced in both treatment groups but not in the placebo-treated group. Significant decreases in faecal *Escherichia coli, Staphylococcus* and *Fusobacterium* counts, but not in *Pseudomonas* and *Enterococcus*, and significant decreases in ammonia, endotoxin, ALT and bilirubin (original level 252±182) were observed in the Synbiotic 2000-treated group (84±65, p<0.01) and in the fibre-treated group (110±86, p<0.05), while it remained unchanged in the placebo group. The improvements in liver function were accompanied by significant improvements in psychometric tests and in the degree of encephalopathy.

In a follow-up study by the same group of investigators 30 patients with liver cirrhosis were randomized to receive either Synbiotic 2000 or placebo (crystalline cellulose) for 7 days (*63*). Viable fecal *Lactobacillus* sp. counts, Child-Pugh class, plasma retention rate of indocyanine green (ICG_{R15}), whole blood tumour necrosis factor alpha (TNF-α) mRNA and interelukin-6 (IL-6) mRNA, serum TNF-α, soluble TNF receptor (sTNFR)I, sTNFRII and IL-6 and plasma endotoxin levels were measured pre- and post-treatment: synbiotic treatment was associated with significantly increased faecal *Lactobacillus* counts and significant improvements in plasma retention rate of ICG_{R15} and stage of liver disease (Child-Pugh classification). No significant changes in any study parameter followed placebo treatment, but significant increases in whole blood TNF-α mRNA and IL-6 mRNA, along with serum levels of soluble TNF receptors sTNFRI and sTNFRII were observed in the Synbiotic 2000-treated patients. TNF-α and IL-6 levels correlated significantly, both at baseline and post-synbiotic treatment. Synbiotic-related improvement in ICG_{R15} was significantly associated with changes in IL-6, both at mRNA and protein levels, and unrelated to plasma endotoxin values. It was concluded that even short-term synbiotic treatment can significantly modulate gut flora and improve liver function in patients with cirrhosis. The observed benefits seemed unrelated to reduction in endotoxaemia, but could be mediated, at least in part, by treatment-related induction of IL-6 synthesis by TNF-α. These results offer great hope that synbiotic treatment of patients on waiting list for liver transplantation might prevent septic episodes, improve liver function, and promote successful outcome of surgery.

Sixty-six patients waiting for orthotopic liver transplantation were randomized to either receive Synbiotic 2000 or synbiotic composition only with fibres. The treatment started already on the day before surgery and continued for 14 days after the surgery. During the first postoperative month only one patient in the Synbiotic 2000-treated group (3 %) showed signs of infection (urinary infection) compared to 17 out of 33 (51 %) in the patients supplemented with only four fibers (*64*). The infecting organisms in the Synbiotic-treated group were *Enterococcus faecalis* in 1 patient and in the fibre-treated group *Enterococcus faecalis/faecium* in 11, *Escherichia coli* in 3, *Enterobacter cloacae* in 2, *Pseudomonas aeruginosa* in 2 and *Staphylococcus aureus* in 1 patient (Table 3). The use of antibiotics was on average (0.1±0.1) day in the Synbiotic-treated patients and (3.8±0.9) days in the fibre--treated group.

Table 3. Isolated bacteria in patients undergoing liver transplantation and treated with Synbiotic 2000 or fibres

Isolated bacteria	Synbiotic-treated	Fibre-treated
Enterococcus faecalis	1	11
Escherichia coli	0	3
Enterobacter cloacae	0	2
Pseudomonas aeruginosa	0	2
Staphylococcus aureus	0	1
Total	1	18

Inflammatory bowel disease

Daily rectal instillations with Synbiotic 2000 reconstituted in saline were given to ten patients with distal colitis during 2 weeks. One patient withdrew after one week, the remaining patients showed dramatic improvements in various disease scores during the 3 weeks of observation; episodes of diarrhoea (2.4⇒0.8), visible blood in stool (2.2⇒0.8), nightly diarrhoea (0.5⇒0), urgency (1.9⇒1.0) and consistency of stool (1.1⇒0.8) (*65*). Two patients reported significant bloating and wind but no other adverse or side effects were reported.

Treatment-Resistant Conditions

Treatment with synbiotics has not this far improved the conditions of disease in two groups of patients: (*i*) inflammatory bowel disease – Crohn's Disease (CD): after an initial treatment with infliximab, sixty-three patients were randomized to receive daily either Synbiotic 2000 or crystalline cellulose as placebo (*66*). Median time to relapse was 9.8 and 10.1 months, respectively. In a second study, following surgery the patients were supplemented with either Synbiotic 2000 or crystalline cellulose as placebo. Seven patients in the synbiotic-treated group and two in the placebo group completed the scheduled 24-month treatment (*67*). No differences were observed between the two groups either in endoscopic findings or the rate of clinical relapse. The so-called Rutgeerts scores were after three months of treatment 0.6±0.8 in the synbiotic-treated group and 0.8±1 in the placebo group (NS); (*ii*) general intensive care patients: two large studies have been performed in a general intensive care population; one with Synbiotic 2000 and one with Synbiotic 2000 Forte. Synbiotic 2000 (40 billion LAB) was given to 162 patients and in the synbiotic composition only with fibres to 168 patients. No difference was observed in mortality or in multi-organ dysfunction (*68*). In another study, 130 patients were supplemented with Synbiotic 2000 Forte (2×400 billion LAB) twice a day throughout the whole ICU stay and compared to 129 patients supplemented with a cellulose--based placebo. No statistical difference was demonstrated between the groups in the incidence of VAP (9 and 13 %, p=0.31). The rate of VAP per 1000 ventilator days was 13 and 14.6 (p=0.73) and hospital mortality 27 and 33 % (p=0.32), respectively (*69*).

Final Remarks

Thousands of factors are important to maintain health and to cure disease. This might explain why single drug pharmacy fails both to prevent a disease and to cure it, especially when chronic. Human innate immunity for a proper function much depends on continuous access to bacteria and plants. Using probiotics in combination with plants and their active ingredients remains an attractive approach for prevention and treatment of various acute and chronic diseases. Ten-year-old studies in the United States demonstrate an 83 % reduction in the rate of coronary heart disease (*70*), a 91 % reduction in diabetes in women (*71*), and a 71 % reduction in colon cancer in men (*72*) in patients adhering to what is regarded as an

'healthy lifestyle': no use of tobacco, moderate use of alcohol, regular physical exercise, and eating a diet low in animal fat, low in refined carbohydrates, and rich in fresh fruits and vegetables (if raw also rich in lactic acid bacteria) and fish, a diet often referred to as Mediterranean diet.

Crohn's disease, as an example, has, despite vigorous attempts over the years, remained most resistant to therapy, pharmaceutical as well as probiotic treatments – see recent review (*73*). Solid observations suggest that several Th1-mediated autoimmune diseases, such as multiple sclerosis, type 1 diabetes, rheumatoid arthritis and Crohn's disease are associated with low vitamin D status, and a recent study demonstrates strong molecular links between vitamin D deficiency and the genetics of Crohn's disease (*74*). Crohn's disease is also strongly associated with lack of minerals, especially calcium and magnesium. Hypomagnesaemia is known to be strongly associated with increased systemic inflammation, manifesting in leukocyte and macrophage activation and increased production of inflammatory cytokines and acute phase proteins. A recent study has demonstrated a much deranged microbiota in experimental animals with induced hypomagnesaemia (*75*). It is certainly too much to request that pre-, pro-, or synbiotics make a difference in such conditions unless the underlying defects in metabolism and immune functions are corrected.

Inflammation is 'the mother of disease' and much associated with the food we eat (*76,77*). As pointed out above, numerous factors contribute to the deranged innate immune system and the sustained exaggerated inflammation. Numerous changes are also required to permanently control the hyper-inflammation and subsequent disease. Supply of pre-, pro-, and synbiotics is a strong tool for such corrections of immune functions and resistance to disease. However, in most instances also other measures are necessary, including reduction in the intake of pro-inflammatory molecules (*2,78*), and also substantial intake of anti-inflammatory food ingredients such as turmeric/curcumin (*79,80*).

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